Design, Synthesis and *In vitro* Antioxidant, Antitumor and Antimicrobial activity of Some Novel 2,3-disubstituted Quinazoline-4(3H)-One Derivatives.

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai.

In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY

(Pharmaceutical Chemistry)

April – 2012

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPATTI ROAD,

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Submitted by

T.ARAVAZHI

Under the guidance of

Mrs. S. HURMATH UNNISSA, M. Pharm.

Assistant Professor,

Department of Pharmaceutical Chemistry

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CERTIFICATE

This is to certify that the dissertation work entitled ***"Design, Synthesis and In vitro Antioxidant ,Antitumor and Antimicrobial activity of some Novel 2,3-disubstituted Quinazoline-4(3H)-one derivatives"*** submitted by **Mr.T.Aravazhi** is a bonafide work carried out by the candidate under the guidance of **Mrs.S.Hurmath Unnissa M.Pharm,** Assistant Professor, to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year **2011-2012**.

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Principal.

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Mrs .S. HURMATH UNNISSA, M.Pharm.,
Assistant Professor,

Dept of pharmaceutical chemistry.

DECLARATION

I do hereby declare that the dissertation work entitled ***"Design, Synthesis and In vitro Anti oxidant, Antitumor and Antimicrobial activity of some Novel 2,3-disubstituted Quinazolin-4(3H)-one derivatives*** "submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** at the Department of Pharmaceutical Chemistry was done by me under the guidance of **Mrs.S.Hurmath Unnissa**, **M.Pharm,** Assistant Professor, at the Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore, during the academic year 2011-2012.

T.Aravazhi

EVALUATION CERTIFICATE

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Internal Examiner **External Examiner**

Convener of Examinations

Examination Center: KMCH College of Pharmacy,
Coimbatore.

Date :

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This dissertation entitled" ***"Design, Synthesis and In vitro Antioxidant, Antitumor and Antimicrobial activity of some Novel 2,3-disubstituted Quinazoline-4(3H)-one derivatives***" would not have been feasible one would not have been a feasible one without the grace of god almighty who gave me moral till the completion of my project

First and foremost I am extremely beholden to my esteemed guide **Mrs. S. HURMATH UNNISSA, M.Pharm.,** Asst. professor, Dept. of Pharmaceutical Chemistry, for her constant insight, personal advice, countless serenity and pain taking effort in all stages of study.

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Above all I dedicate myself before the unfailing presence of ***GOD*** and constant love and encouragement given to me by my beloved **Parents, Grandpa, Grandma and Brother** who deserves the credit of success in whatever work I did.

T.ARAVAZHI

ABBREVIATIONS USED

DMSO - Dimethyl Sulfoxide

FTIR - Fourier Transform Infrared Spectrometer

IR - Infrared Spectral analysis

TLC - Thin Layer Chromatography

UV - Ultraviolet and Visible Spectroscopy

NMR - Nuclear Magnetic Resonance

IC_{50} - Inhibitory Concentration Percentage

Std - Standard

Mins - Minutes

Hrs - Hours

Mg - Milligram

Ml - Millilitre

Mm - Millimetre

µg - Microgram

δ - Delta

λ - Lambda

^{0}C - Degree Celsius

% - Percentage

DPPH - 1,1-diphenyl 2-picryl hydrazyl

FRAP - Ferric reducing antioxidant Power

Chapter 1

Introduction

INTRODUCTION

One of the Prime motives of a medicinal chemist is service to medicine Medicinal chemists may have been stimulated by the need of the sick for drugs**.** By contributing

therapeutic agents, medicinal chemists have made possible, some of the proudest and most spectacular achievements of human and veterinary medicine.

The discovery of the medicinal usefulness of a compound has always stimulated inquiry in to the chemical reactions and improved methods of preparation of similar substances. This introduces new dimension in the field of synthetic chemistry like any artistic and creative activity[1].

Medicinal chemistry is an interdisciplinary science. It has been stated that 'Medicinal Chemistry concerns the discovery, the development, identification and interpretation of the mode of action of biologically active compounds at the molecular level. Evidently it touches all branches of chemistry and biology[2].

The biological direction has added the roles of enzymologist and molecular biologist to the group of research scientists working under a medicinal chemical designation. The physiochemical direction has required that a quantum mechanician, Spectroscopist, and bio-pharmacist be included. Attempts to correlate or reconcile the results of bio-chemical measurements with physiochemical calculations also occupy the attention of medicinal chemists[2].

Medicinal chemistry, according to Burger, tries to be based on the ever-increasing hope that biochemical rationales for drug discovery may be found. In contrast, he described pharmaceutical chemistry with modification of structures having known physiologic or pharmacologic effect and with analysis of drugs[3].

CANCER:

Normal cells in our body follow an orderly path of growth, division and death. Cancer is a class of diseases characterized by out-of-control cell growth which harms the body by forming lumps or masses of tissue called tumors. Tumors are invasive, aggressive and mostly metastatic. Tumors that stay in one spot and show limited growth are called benign which can often be removed, and, in most cases, they do not come back. Also, cells in benign tumors do not spread to other parts of the body. Cancer is, thus, the result of cells that uncontrollably grow and do not die.

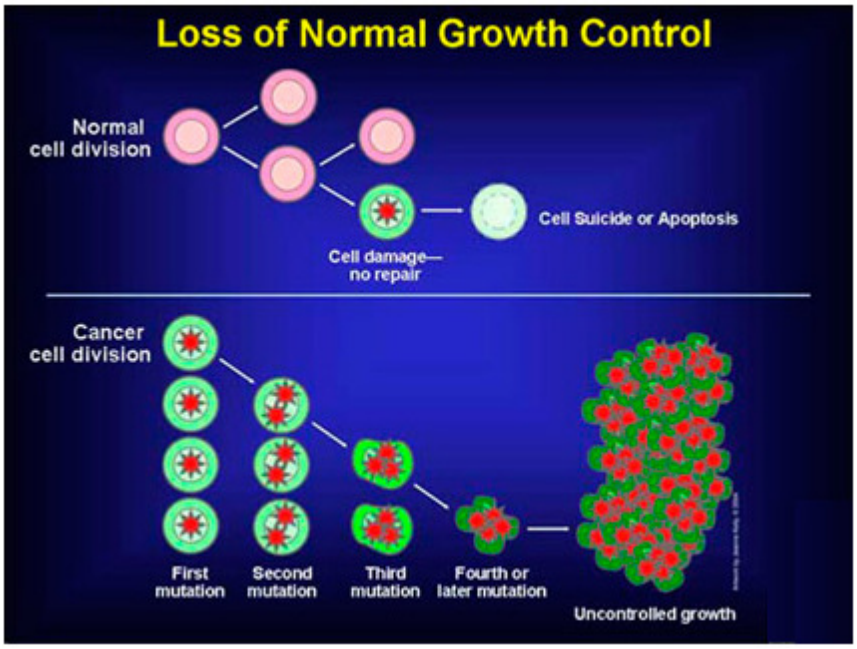


Fig 2. Pathology of cancer

CAUSES OF CANCER

- Exposure to carcinogens
- Ionising radiations
- Infections
- Hormonal imbalances
- Immune system dysfunction
- Heredity

TYPES OF CANCER

- **Carcinoma** - cancer of the skin or tissues that line or cover internal organs
- **Sarcoma** - cancer that starts in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue
- **Leukemia** - cancer that starts in blood-forming tissue such as the bone marrow

- **Lymphoma and myeloma** - cancers that begin in the cells of the immune system
- **Central nervous system cancers** - cancers that begin in the tissues of the brain and spinal cord
- **Adenoma** - cancers that arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues

TREATMENT

- Surgery
- Radiation therapy
- Immunotherapy
- Hormone therapy
- Gene therapy
- Chemotherapy

CANCER CHEMOTHERAPY

Chemotherapy refers to the treatment of an ailment by chemicals especially by killing micro-organisms or cancerous cells. Anti neo plastic drugs are used in cancer chemotherapy. A single "cure" for cancer has proved elusive since there is not a single type of cancer but more than hundred different types of cancer. Though the available anticancer drugs have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells, their toxicity to normal rapidly growing cells in the intestinal and bone marrow areas and the problem of resistance pose a great problem. For this reason cancer

chemotherapy may consist of using a combination of several drugs for varying lengths of time.

Categories of Chemotherapy Drugs

Based on their mechanism of action, chemotherapy agents can be divided into three main categories as follows:

- **Drugs that stop the synthesis of DNA building blocks**

  DNA building blocks are folic acid, heterocyclic bases, nucleotides etc. The agents that work to block some step in the formation of these building blocks come under this category. Examples of drugs in this class include methotrexate, fluorouracil, hydroxyurea, mercaptopurine etc.

- **Drugs that directly damage the DNA**

  These agents chemically damage DNA and RNA by disrupting replication of the DNA or by the manufacture of nonsense DNA or RNA. Examples of drugs in this class include cisplatin, antibiotics – daunorubicin, doxorubicin, etoposide etc.

- **Drugs that affect the synthesis or breakdown of the mitotic spindles**

  These drugs disrupt the formation of these spindles and therefore interrupt cell division. Examples of drugs in this class include mitotic disrupters such as Vinblastine, Vincristine, Paclitaxel etc.

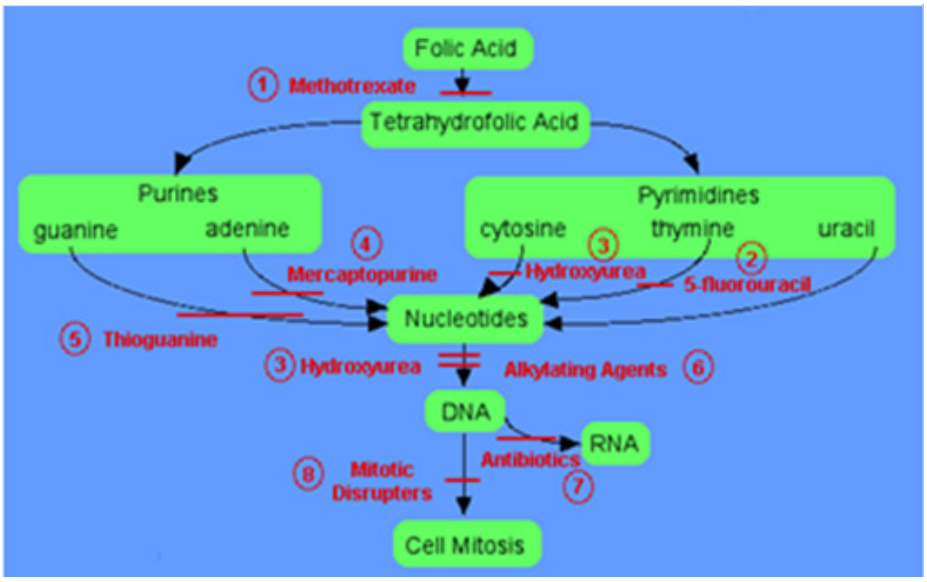


Fig 3. Mechanism of action of some anticancer drugs

ANTIOXIDANT

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD),glutathione peroxidase (GPx), and catalase)and non-enzymatic antioxidants (e.g.,ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage and health problems. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources. These natural plant antioxidants can therefore serve as a type of preventive medicine. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease. However, synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis.

Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids and tannis), have diverse biological effects, such as anti-inflammatory, anti-carcinogenic and anti atherosclerotic effects, as a result of their antioxidant activity.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or poly phenols.

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Reactive oxygen species (ROS). Capable of causing damage to DNA, has been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age[4]. In low concentrations, synthetic antioxidants are also in use for many industrial processes e.g. inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers. They exert their effects by scavenging or preventing the generation of ROS[5] which can protect the formation of free radicals and retard the progress of many chronic diseases including cancer, neurodegenerative, inflammation and cardiovascular diseases[6,7]

Heterocyclic compounds:

Heterocyclic rings, which have been reason for the activity of most of the drugs of natural origin leads to the discovery of many synthetic drugs possessing the heterocyclic rings and their fused analogs represent an important class of heterocyclic compounds exists in numerous natural products displaying a wide range of biological and pharmaceutical activities. On intensive research heterocyclic derivaties continue to yield new medicinal agents[8].

Quinazoline:

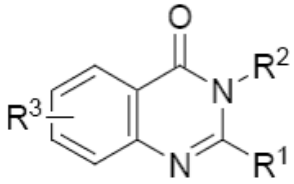
Quinazoline is a bicyclic compound consisting of a pyrimidine system fused at 5, 6 with benzene ring having broad spectrum of medicinal values. It is a compound made up of

two fused six membered simple aromatic rings, benzene and a pyrimidine ring with keto group at 4th position. Its chemical formula is $C_8H_4N_2O$. It is a yellow and crystalline compound. Any derivative of it may be described as Quinazolinone compound. 4(3H)-Quinazolinone with 3-substitution and various substituted phenyl ring moieties, bridged phenyl rings, heterocyclic rings and aliphatic systems were reported to possess antimicrobial properties.

Quinazoline ring is a versatile lead molecule which has been investigated widely in medicinal chemistry due to its important pharmacological activities. These involve analgesic[9], anti-inflammatory[10], antihypertensive[11], sedative and hypnotic[12], antihistaminic[13], antitumor[14], antimicrobial, anticonvulsant[15], enzyme inhibition activity[16] and many other activities.

The new and improved methods for the construction of the 4(3H)-Quinazolinone and Quinazoline skeletons with a particular emphasis on the 2-substitued and 2,4-disubstituted analogues.

Quinazolin-4(3H)-one is 4 oxo-1,3-bezopyrimidine and the detailed chemistry are discussed in textbook of heterocyclic chemistry.



From literature review it is known that most of the Quinazolin-4(3H)--ones having substitution at C-2 and N-3 positions, posses anti microbial activity.

4(3H)-Quinazolinones and their derivatives constitute an important class of heterocyclic compounds. They occupy an important position in medicinal chemistry, presenting a wide range of bioactivities. Many of them display anti-microbial, anti-tubercular, anticancer, anti-HIV, anti-fungal, anti-inflammatory, anticonvulsant, antidepressant, hypolipidemic, antiulcer and analgesic or immunotropic activities and are also known to act as thymidylate synthase, poly (ADP-ribose) polymerase (PARP) protein tyrosine Kinase inhibitors and dihydrofolate reductase inhibitors. As pesticides, they are used as insecticides, fungicides and antiviral agents such as TMV, CMV inhibitors. It is found in many bioactive natural products and looking at the biological significance of Quinazolinone nucleus it was thought to design and synthesize new derivatives of it.

Quinazolin-4(3H)-one constitute a unique group of compound due to the simultaneous presence of three characteristic feature

- Enormous synthetic possibilities are offered by the presence of acetanthranils, often serve as starting material for more complex compounds via cyclization, condensation etc.,
- Stability of the nucleus and Wide range of biological activity

The synthesis of both Quinazolinones and Quinazolines will be classified into five categories, based on the substitution patterns of the ring system.

- 3-Substituted-4(3H)-Quinazolinones
- 4-substituted Quinazolines
- 2,3-disubstituted-4(3H)-Quinazolinones
- 2,4-disubtituted-4(3H)-Quinazolinones and Quinazolines
- Many of this 2, 3-disubstituted derivatives are of material importance.

Combinatorial Approaches to Quinazolinones

2,3-disubstituted Quinazolinones can be synthesized via benzoxazinones and benzoxadiones.

The intermediate (4H)-3, 1-benzoxazinones were reacted with amino substituted compound and get the desired product.

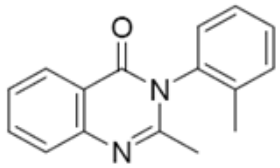
Using Quinazolinones it would be possible to introduce a variety of substitution in the core structure in the areas shown in figure**.** It would be possible to alter the length and nature of the 2-alkyl chain (R^1), change the functionality at the 3-position (R^2) and to introduce substitution into the carbocyclic ring (R^3).

Fig: 1.1 Structural areas of Quinazolinones that can be altered for SAR study.

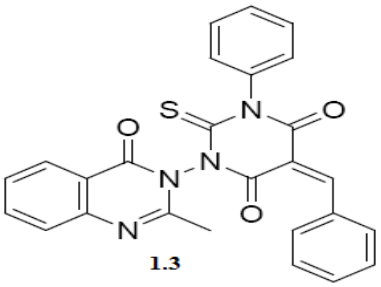
These alterations could be achieved individually or in combination and their effects assessed within biological assays. This structure activity relationship (SAR) study would thus provide a number of new molecules that can be assayed for their immune modulatory properties. It was also intended to use the Quinazolinone structure as a surrogate structure, expecting that alterations will mimic the relative equivalents in the original 4-quinolone system. An example of this would be observing the effect of altering the length of the side

chain and seeing if the activity rises or falls. It would be of interest to see if the change in activity parallels the 4-quinolone analogues. A number of Quinazolinones of varying complexity in structure have been reported to have a range of medicinal properties.

For example, Methaqualone **1.2** has sedative effects, 2-alkyl/aryl-3- arylhydrazino-4(3*H*)-Quinazolinones **1.3** possess antibacterial activity, as well as central nervous system (CNS) effects similar to that of monoamine oxidase inhibitors.

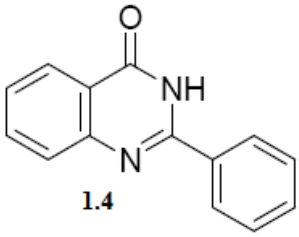


1.2



1.3

Derivatives of 2, 3-dihydro-2-(aryl)-Quinazolinone (DHQZ) **1.4** show some anti-tumor effects and various 2-methyl Quinazolinone analogues have been synthesized that possess anti-folate properties[17].



1.4

Many derivatives of Quinazoline system known so far, keto-Quinazolines also called as Quinazolinones, are the most important compounds. Depending upon the position of the keto or oxo group, these compounds may be classified into two types: 2-(1H) Quinazolinones (or) 1,2-dihydro-2-oxo Quinazolines and 4(3H)-Quinazolines or 3,4-dihydro-oxoquinazolines . These systems exhibit lactam-lactam tautomerism and undergo hydroxy group replacement reactions. 2-Cyano-4(3H)-Quinazolinone was the first Quinazolinone derivative to be synthesized.

2. Brief Account of reactivity of 4(3H)-Quinazolinones:

Reactions associated with tautomeric nature of the Quinazolinones are often quite complex and generally unpredictable. The recorded chemical investigation on the subject is voluminous. The amide linkages in quinazolinones should not be looked on as predominantly

the keto or the enol form but as true keto-enol tautomers, showing reaction characteristic of both the forms.

Quinazolinones are always high melting crystalline solids, insoluble in water and in most organic solvents but soluble in aqueous alkali. They are generally insoluble in dilute acids but are sometimes soluble in concentrated acids. Simple 4(3H)-Quinazolinones, although insoluble in dilute acids, are soluble in 6N hydrochloric acid. 4(3H)-Quinazolinones form stable monohydrochlorides, chloroplatinate, chloroaurates and picrates and their metal salts of silver, mercury, zinc, copper, sodium and potassium.

Stability of the ring system:

The ring system in Quinazolinone is exceedingly stable in oxidation, reduction, hydrolysis reactions and other treatment designed to break the ring. There is no report of degradation of Quinazolinone by simple chemical oxidation.

Aromatization:

When a simple and 2-substituted- 4(3H)-Quinazolinone is heated with an equivalent amount of phosphorous pentachloride in phosphorous oxychloride, the corresponding 4-chloroquinazoline is obtained. If a methyl group is present at 3-position, prohibiting the usual tautomerism, the methyl group is lost during the chlorination.

Alkylation:

The position of alkylation of Quinazolinones is similar to all the aromatic nitrogen heterocyclic systems in which a hydroxyl group is found ortho or para to the nitrogen position. Such compounds exist in tautomeric mixture, the two structures being inter-convertible by the shift of one proton and one pair of electrons. In alkaline solution the ions of such compounds exist as resonance hybrids of the two major forms differing only by the position of two pairs of electrons, as shown. Thus in alkylation of such hydroxyl derivatives of pyridine, pyrimidine and similar heterocycles, the entering group may become attached to either the nitrogen atom, thus giving for instance, an N-alkyl-pyridine or to the oxygen atom, giving an alkoxy pyridine.

Nitration:

4(3H)-Quinazolinone on boiling with nitric acid undergoes substitution to give 6-nitro-4 (3H)-Quinazolinone. On further nitration it has been observed that the second nitro

group enters the 8-position to give 6,8-dinitro derivatives. 2-Substituted-4(3H)-Quinazolinones were also found to behave similarly, under such conditions.

Reduction:

2,3-Dihydro-3-methyl- 4(1H)-Quinazolinone could be obtained on reduction of 3-methyl-4(3H)-Quinazolinone with Lithium Aluminium Hydride ($LiAlH_4$) in benzene.

Amino acids:

Amino acids are organic compounds, containing an amino group and a carboxyl group. Amino acids are the end product of protein digestion and the basic building blocks from which proteins digestions and the basic building blocks from which proteins are synthesized in the cell.

All proteins are made up of amino acids. Amino acids contains a carbon atom, a free among group (containing nitrogen-NH2) and a carboxyl group (COOH). Amino acids are Amphoteric in reaction and forms salts with both acids and bases. Amino acids are colourless, crystalline substance, soluble in water, easily diffusible and (except glycine) optically active. When the amino and carboxyl groups of amino acids combine acid residue A peptide thus consists of two or more amino acids linked by peptide bonds.

Amino acids are usually classified by the properties of their side chain into four groups. The side chain can make an amino acids a weak acid or a weak base, and a hydrophile if the side chain is polar or a hydrophobe if it is nonpolar . The chemical structures of the 22 standard amino acids , along with their chemical properties, are described more fully in the article on these proteinogenic amino acids.

Zwitterions:

The amine and carboxylic acid functional groups found in amino acids allow them to have amphiprotic properties. Carboxylic acid groups (-COOH) can be deprotonated to become negative carboxylates (-CO2-), and α- amino groups (NH2-) can be protonated to become positive α-ammonium groups (+NH3-). So has net zero charge. This molecular state is known as a zwitterion, from the german zwitter meaning hermaphrodite or hybrid. Below pH 2.2, the predominant form will have neutral carboxylic acid group and positive α-ammonium ion (net charge+1), and above pH 9.4, a negative carboxylate and neutral α-amino group (net charge-1).

Zwitterions have minimum solubility at their isoelectric point and some amino acids (in particular, with non polar side chains) can be isolated by precipitation from water by adjusting the pH to the required isoelectric point.

Amino acids have both a primary amine group and a primary carboxyl group, these chemicals can undergo most of the reactions associated with these functional groups. These include nucleophilic addition, amide bond formation and decarboxylation for the carboxylic acid group.

Amino acids can be benzolyated in aqueous solutions of sodium hydroxide to avoid racemisation and to improve the yield of benzoyl derivative[18].

Chapter 2

Literature Review

LITERATURE REVIEW

BIOLOGICAL ACTIVITIES OF QUINAZOLINES

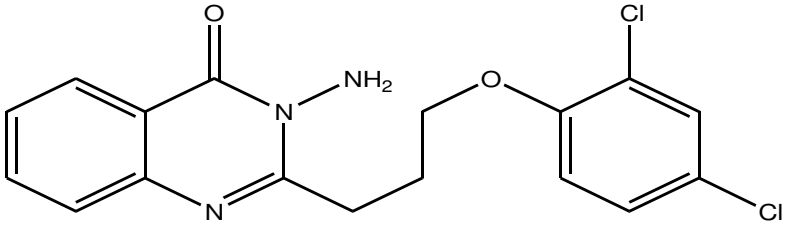
Quinazolinone ring is an aromatic benzopyrimidine system many of it's derivative posses interesting biological activities. These involve analgesic, anti-inflammatory, anti-hypertensive, sedative and hypnotic antihistaminic, antitumour, anti-microbial, anti-convulsant, antitubercular and many other activities.

Most of the biological active Quinazolinones are having substitution at C-2 and N-3 position

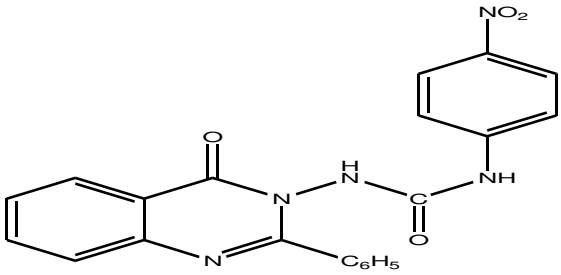
A brief review of pharmacologial activities exhibited by Quinazolinones is present here in.

ANTI-CONVULSANT ACTIVITY:

1. **Hanan Georgey and Safinaz Abbas[19];** A number of 3-substituted-2-(substituted-phenoxymethyl) Quinazoline-4(3H)-one derivatives synthesised .A preliminary evalution of the anticonvulsant properties of the prepared compounds has indicated that some of them moderate activity compared to a Diazepam standard.

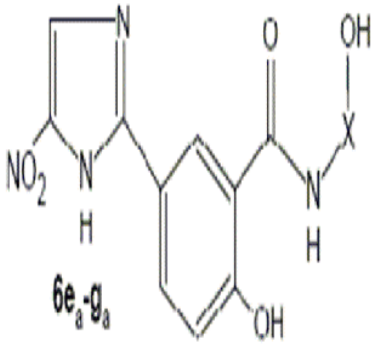


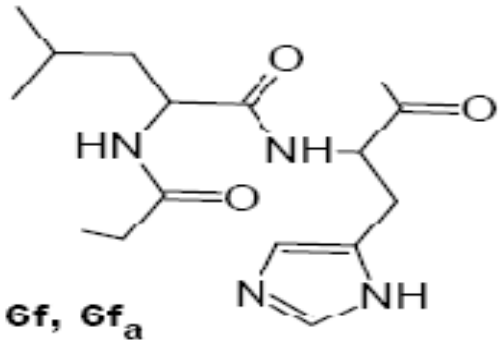
2. **Sushil K. Kashaw and Varsha Kashawa[20];** A new 1-(4-substituted-phenyl)-3-(4-oxo-2-phenyl-4H-Quinazoline-3-yl)-urea were synthesised and screened for anticonvulsant activity.



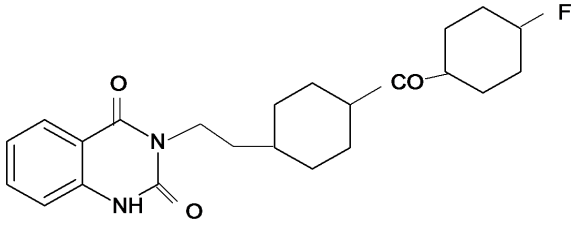
ANTI – HYPERTENSIVE ACTIVITY

3. **Rajiv Dahiya et. al**.[21], has synthesized and carried out the biological activity of peptide derivatives of iodoquinazolinones/nitroimidazoles. Two substituted quinazolinyl/imidazolyl-salicylic acids **5**, **6** were synthesized by the reaction of 6 -iodo-2-methylbenzoxazin-4-one/5-nitroimidazole with 5-aminosalicylic acid (5 -ASA). Coupling of compounds **5** and **6** with different amino acid ester hydrochlorides, dipeptide and tripeptide methyl esters yielded novel quinazolino/imidazolopeptide derivatives. **6f** and its hydrolyzed derivative **6fa** showed good anthelmintic activity against *Megascoplex konkanensis*, *Pontoscotex corethruses* and *Eudrilus eugeniea* at dose of 2 mg ml^{-1}.



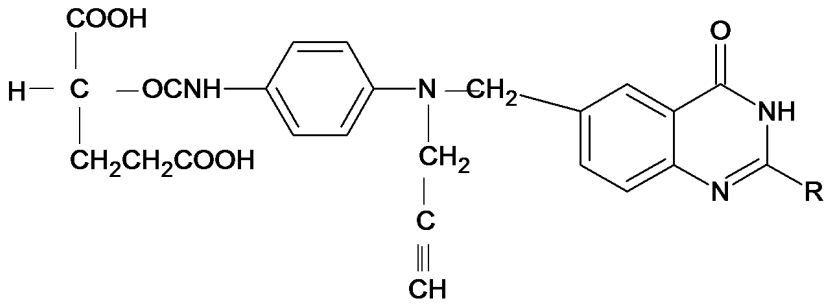


Quinazolinone derivative, ketanserin exhibits anti – hypertensive activity and is an antagonist of 21 – ADRENERSIC AND SEROTENIN S_2 RECEPTORS



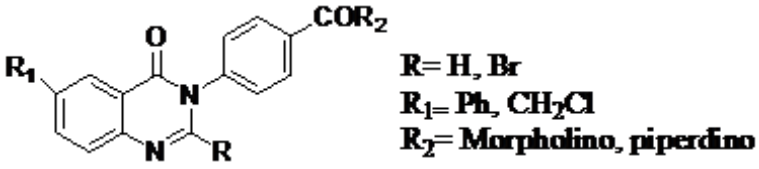
ANTI – CANCER ACTIVITY

4. **Jones and co-workers**[22] has reported that {N- [4-(2-amino – 3,4 – dihydro-4- oxo-6-quinazolyl) Methyl] –N-Prop-2-Ynyl amino] benzoyl}- L –Glutamic acid has been found to be a selective thymidylate synthetase (TS)[(4)] inhibitors. It has shown encouraging anti-tumor activity against breast and ovarian cancers in recent clinical trials. The compound has shown 50 – fold improvement over in therapeutic index.

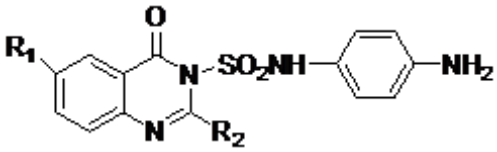


ANALGESIC AND ANTI – INFLAMMATORY ACTIVITY

5. **Rita Nigam et al**[23]**.,** has prepared quinazolin -4 (3H) ones for anti- inflammatory activity.

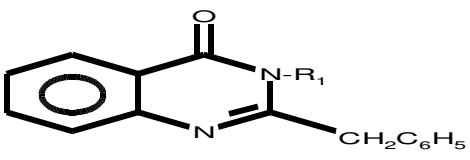


6. **Hithari, etal**[24]**.,** has reported synthesis of 6 substituted -2-alkyl-3-(4-aminobenzenesulphonamido) quinazolin-4-(3H)-one and its anti- inflammatory activity.

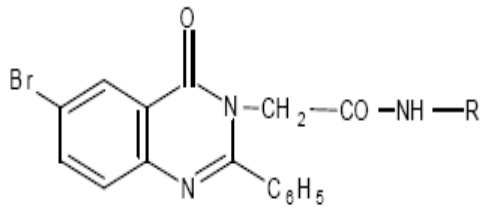


ANTIMICROBIAL ACTIVITY

7. PRADEEP MISHRA, SANMATHI K. JAIN AND SANDEEP JAIN[25,] synthesized some new Quinazolin 4(3H) ones (II) as possible antimicrobial agents.

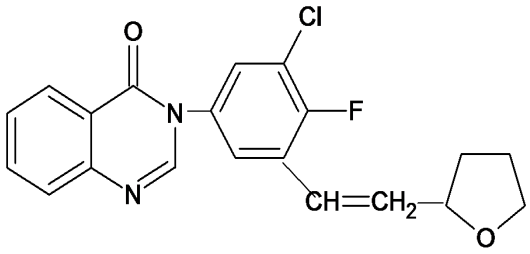


8. **CH.RAJVEER et. al**.[26], has carried out for the synthesis of 2-(6-bromo-2-phenyl-4 oxoquinazolin-3(4H)-yl)-N-substituted acetamides and 1-Amino-5-(6- bromo-3, 4-dihydro-2-phenyl-4-oxoquinazolin-3yl) methyl-1, 3, 4-triazin-2-thiol to carry out their pharmacological activities. The synthesized compounds were screened for their anti-bacterial activity, anti-inflammatory activity and analgesic activity by standard methods. The compound shows Pharmacological activities in comparison with the standard.

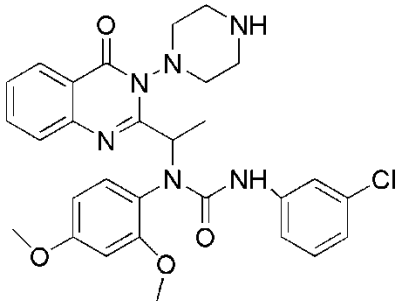


IV(a-e)

9. **B.Shivarama Holla et al**[27]**.,** describes the synthesis and characterization of some fluorine containing arylfuryl vinyl quinazolinones as possible antibacterial agents.



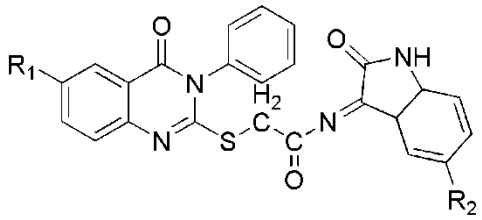
10. **Remy C.Lemoine et al (2004)**[28]**,.**reported the discovery of a series of quinazoline based fungal efflux pump inhibitors by high-throughput screening for potentiation of flucanazole. Attempts to improve the aqueous solubility of screening hits led to improved physical properties and activity against clinically-relevant candida spp.



3-(3-chlorophenyl)-1-(1-(3,4-dihydro-4-oxo-3-(piperazin-1-yl)quinazolin-2-yl)ethyl)-1-(2,4-dimethoxyphenyl)urea

ANTIVIRAL ACTIVITY

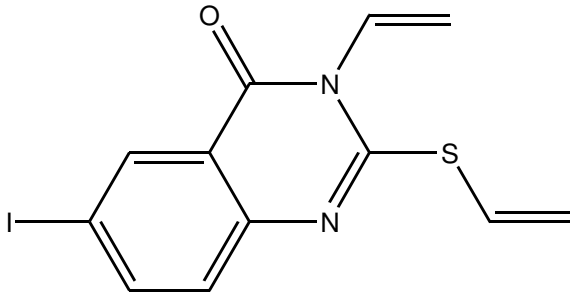
11. **Nilgun Karali et al[29] ,** reported new esters and hydrazides were synthesized from 6-methyl/fluro-3-phenyl-4(1H, 3H)-quinazolinone-2-thiones. Compound was confirmed moderately active against HIV-1.



R1 ,R2= H,Br

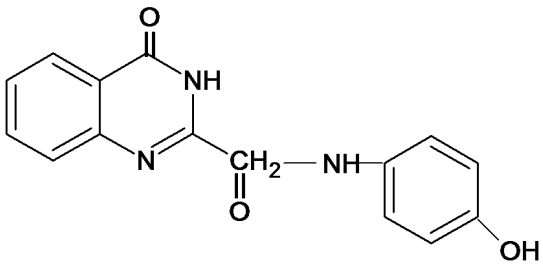
ANTI-TUBERCULAR ACTIVITY;

12. A series of 21 new 2-alkylthio-6-iodo-3-substituted-quinazolin-4-one derivatives was prepared andscreened for their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* strain H Rv, using the 37 radiometric BACTEC 460-TB methodology.Active compounds were also screened by serial dilution to assess toxicity to a VERO cell line.



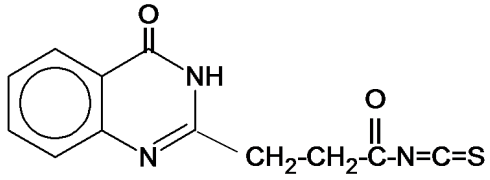
MISCELLANEOUS

13. **Shukla and Rastigum[30]**, has synthesized a series of 2-substitued quinazoline4(3H)-ones and tested for their anti helmintic activity against Brugia Pahangi and Hymenoepis nanc in birds and rates Compound 2 – [3-substituted amino methyl-l-4'-hydroxy phenyl] quinazolin- 4(3H) – one (43) is found to be most active member of the series, showing 65% clearance of H. an infestation at a dose 250mg/Kg for 3 days.

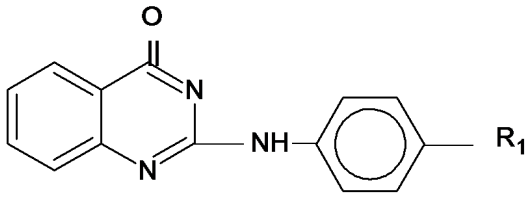


N = 4 -[P-Chlorophenyl] -1- piperazinyl

14. **MS Amine et al[31].,** has reported the uses of quinazolin-2-[-Propionoyl] isothio cyanate]-4 ne as a building block in synthesis of some heterocyclic compounds of expected biological activities.



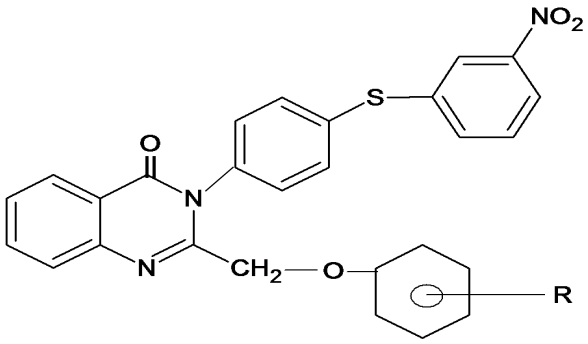
15. **PSR Reddy et al[32],** has reported A facile synthesis of 1 – ary 1-4 –{isopropylidene amine /methyl -4-(3h) – oxoquinazolin -2-4}] azetidin-2 ones.



R = H R= NH_2

R = CH_3

16. **Shukla, et al[33].,** has reported antimalarial activity of 2- aryloxymethyl-3[p'-nitrophenylthio) phenyl] quinazoline-4(3H) one which is having ED^{50} <10mg. Kg. and ED 90>10mg/Kg.



Chapter 3

Aim and Objectives

AIM OF THE PRESENT WORK

Cancer is a class of diseases characterized by out-of-control cell growth which harms the body by forming lumps or masses of tissue called tumors. Tumors are invasive, aggressive and mostly metastatic. Cancer is life threatening due to cells that uncontrollably grow and do not die. Under stress, our bodies produce more reactive oxygen species (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid, tocopherol, glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage and health problems. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases.

Microorganisms like bacteria and fungi cause many infections like meningitis, ottis media, pneumonia, cholera, food poisoning, urinary tract infections, aspergillosis, candidose etc. Many of these diseases are fatal if untreated, and treatment has been complicated by the resistance of the microorganisms to the widely used drugs. To combat the problem of resistance newer drugs are needed.

Numerous research has shown that the Quinazoline nucleus possesses potent activity against human cancer particularly by killing the specific tumor cells. The nucleus has also been

reported to have anti oxidant and potent antimicrobial activities. These observations gave us a great impetus to the search for potential biological active drugs carrying 2,3-disubstituted Quinazoline-4(3H)-one in combination with amino acids and sulfonyl hydrazides, hoping to add some synergistic biological significance to the target molecules to get potent anti oxidant, anti tumor and antimicrobial agents.

Quinazolinone ring is an aromatic benzo pyrimidine system. The Quinazolinone nucleus have attracted the attention of medical chemist due to a wide range of biological activity exhibited by them.

Quinazoline 4(3H) one constitute a unique group of compound due to the simultaneous presence of three characteristic feature

1. Enormous synthetic possibilities are offered by the presence of acetanthranils, often serve as starting material for more complex compounds via cyclization, condensation etc.,
2. Stability of the nucleus and
3. Wide range of biological activity.

Structural modifications involving variations in the nature of groups in the 2 and 3 positions and substitution into fused benzene ring have lead to a large number of derivatives that are biologically active.

Interests in quinazolines as anticancer agents have further increased since the discovery of Raltitrexed and Thymitaq proved to be Thymidylate enzyme inhibitors in cancer treatment.

In order to produce potent new leads for anticancer drugs, a new series of 2,3 di substituted Quinazolin4(3H)one analogue have been designed by fusing with some biologically friendly amino acids and some sulfonyl hydrazide the structural features which are believed to enhance tumor inhibition activity.

Amino acids conjugated Quinazolinones are reported to have a greater binding affinity, enhanced anti tumor and antimicrobial activity[34]

Amino acids will minimize the side effects of the metabolite of the parent compound upon metabolism in the body and enhance the solubility of the synthesised candidates when it is incorporated into pharmacologically active Quinazolinone moiety[34].

. **The objective of the following work can be summarized as follows** :

- Synthesis of some Novel 2, 3-disubstituted-4(3H)-Quinazolinone derivatives.

- Characterization of the synthesized compound using the various analytical and spectral techniques.
- Screening for Anti Oxidant activity by DPPH & FRAB method.
- Screening for *In vitro* Anti tumor activity against human cervical cancer cell line (HeLa) by MTT assay method.
- Screening for the Antimicrobial activity against various microorganisms using Disc diffusion method.

Chapter 4

Experimental Procedure

EXPERIMENTAL

Scheme -1:

Step-1:

Synthesis of 2-chloro benzyl 1, 3-benzoxazin-4-one:

A mixture of Para chloro phenyl acetic acid (0.06 mol) and phosphorous penta chloride (0.06 mol) was triturated to get chloro phenyl acetyl chloride. Anthranilic acid (0.06 mol) was dissolved in 30ml of anhydrous pyridine by stirring slowly at room temperature, cooled to 0^0 c and a solution of chloro phenyl acetyl chloride in anhydrous pyridine 30ml was added to this solution slowly with constant stirring.[35] When the addition was complete the reaction mixture was stirred for half an hour mechanically at room temperature and set aside for 1 hr. The pasty mass obtained was diluted with water and treated with aqueous sodium bicarbonate to remove the un reacted acid when effervescence ceased, The solid material was filtered off and washed with water to remove the inorganic material adhered pyridine. The crude benzoxazinone thus obtained was dried and re-crystallized from dilute ethanol.

Step-2:

Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazolinone-3(4H)-yl) benzoic acid:

10gm of 2-chlorobenzyl 1,3-benzoxazin-4-one was added to a mixture of 4- amino benzoic acid (6.31gm) and glacial acetic acid(30ml) and the mixture was refluxed under anhydrous

condition for 6hrs. After then added ice cold water into it and the crude the product was filtered and dried. The crude product was recrystallised from 1,4-dioxane..

Step-3:

Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazolinone-3(4H)-yl) benzoyl chloride:

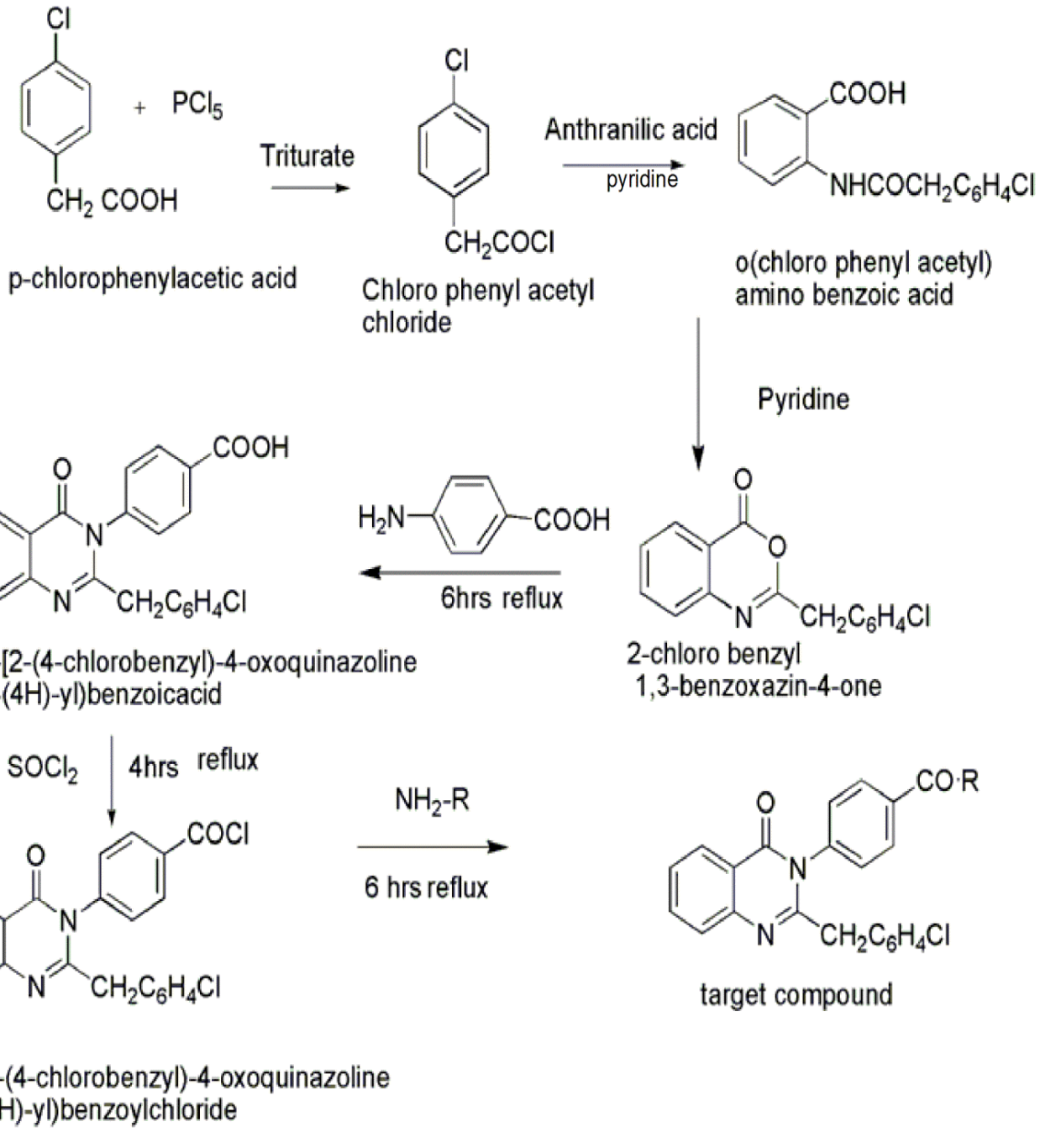
A solution of 4-[2-(4-chlorobenzyl)-4-oxoquinazolinone-3(4H)-yl) benzoic acid (7.03gm) in 1,4-dioxane (20ml) was placed in a 250ml flask fitted with a condenser . Thionyl chloride (2ml) was then added drop wise to the flask using dropping funnel. The mixture was refluxed under anhydrous condition for 4hrs. The excess of thionyl chloride was removed by distillation. The reaction mixture was poured into the 100ml ice cold water and the crude product was filtered and dried. The dried crude product was recrystallised from 1,4-dioxane.

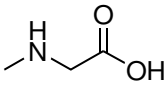
Step-4:

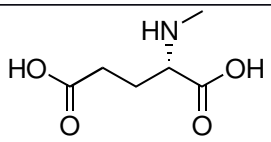
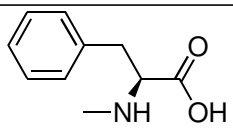
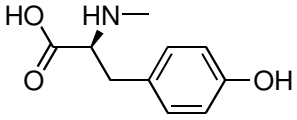
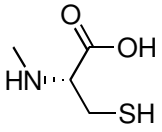
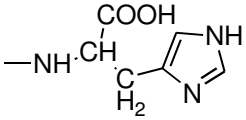
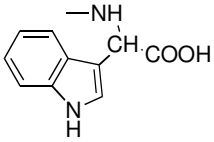
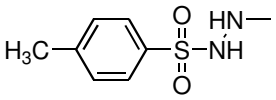
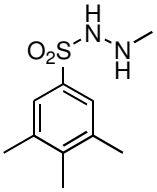
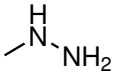
Synthesis of 4-[2-(4-chlorobenzyl)-4-oxoquinazolinone-3(4H)-yl) benzoyl] derivatives

Asolutionof4-[2-(4-chlorobenzyl)-4-oxoquinazoline3(4H)yl)benzoylchloride(4.1gm.0.01mol) in 1,4-dioxane was added to corresponding amino acid (0.01mol) and sulfonyl Hydrazide in 0.1N sodium hydroxide (10ml) and the mixture was refluxed for 6hrs.[36] The reaction mixture was then poured into 1N Hydrochloric acid (50ml) and the crude products were filtered and dried. The dried products were recrystallised from 1, 4-dioxane.

Scheme:



S.NO	Compound code	R
1	C-GY	

2	C-GU	
3	C-PA	
4	C-TY	
5	C-CY	
6	C-HS	
7	C-TP	
8	C-PT	
9	C-T	
10	C-HH	

CHARACTERIZATION

MELTING POINT: [37]

Melting points of the synthesized compounds were determined in a one end fused capillary tube method by using Thermonic Model –C-LMP- 1 CAMPVEEL melting point apparatus, and were uncorrected..

THIN LAYER CHROMATOGRAPY:

Thin layer chromatographic analysis was carried out for all synthesized compounds by using silica gel G (0.5mm thickness) coated over glass plate (12 x 20 cm) as stationary phase, Acetonitrile: EthylAcetate: Methanol (1:1:1) as mobile phase and the spot was visualized by iodine vapor.

ULTRA VIOLET SPECTRAL ANALYSIS:

The maximum absorbance or λ max of synthesized compounds was determined in the concentration of 0.01% w/v in DMF by using shimadzu 2000 ultraviolet spectrophotometer.

INFRARED SPECTRAL ANALYSIS: [38]

The structures of the synthesized compounds were elucidated by JASCO FT-IR spectrophotometer in KBr disc. The IR value is measured in cm^{-1}.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY:

The structures of the synthesized compounds were to be elucidated by Bruker 300 MHz FT- NMR using TMS (Tetramethylsilane) as internal standard. The PMR (Proton Magnetic Resonance) spectroscopic values are measured in δ ppm in DMSO.

MASS SPECTROSCOPY:

The structures of the synthesized compounds were to be elucidated by MS (EI) JEOL GC MATE 700EV Mass spectroscopy. The mass spectroscopic values are measured in m/e ratio.

PHYSICO-CHEMICAL PARAMETERS OF SYNTHESISED DERIVATIVES

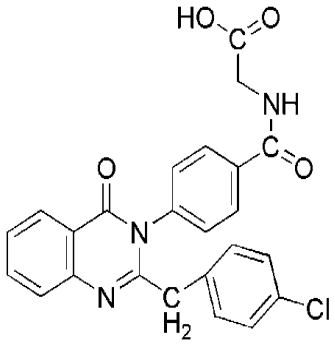
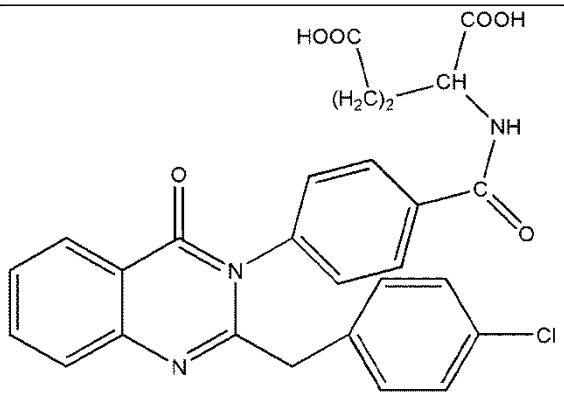
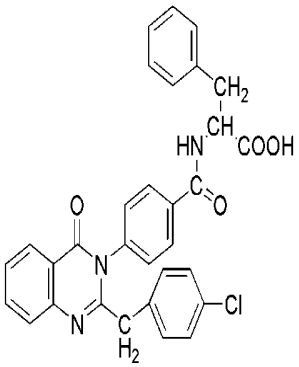
S.No	Compound Code.	Molecular formula	Molecular weight	Melting point	Rf value	solubility	Percentage yield
1.	C-GY	$C_{24}H_{18}ClN_3O_4$	447.87	195	0.34	Ethanol/DMSO	72%
2.	C-GU	$C_{27}H_{22}ClN_3O_6$	519.93	205	0.58	Ethanol/DMSO	78%

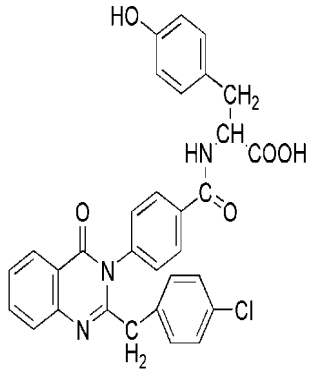
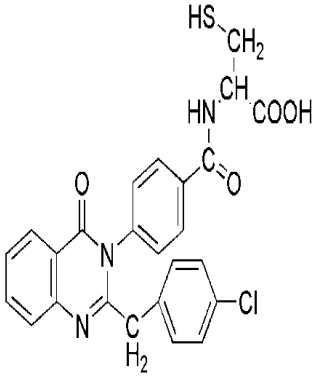
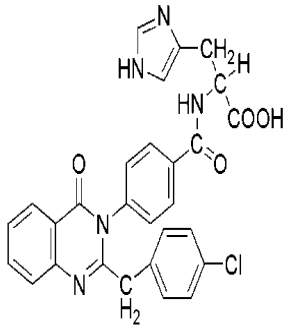
3.	C-PA	$C_{31}H_{24}ClN_3O_4$	537.99	246	0.38	Ethanol/DMSO	69%
4.	C-TY	$C_{31}H_{24}ClN_3O_5$	553.99	256	0.72	Ethanol/DMSO	82%
5.	C-CY	$C_{25}H_{20}ClN_3O_4S$	493.96	225	0.68	Ethanol/DMSO	74%
6.	C-HS	$C_{28}H_{22}ClN_5O_4$	527.96	198	0.44	Ethanol/DMSO	86%
7.	C-TP	$C_{33}H_{25}ClN_4O_4$	577.03	270	0.32	Ethanol/DMSO	93%
8.	C-PT	$C_{29}H_{23}ClN_4O_4S$	559.04	246	0.62	Ethanol/DMSO	86%
9.	C-2	$C_{31}H_{27}ClN_4O_4S$	587.06	298	0.74	Ethanol/DMSO	92%
10.	C-HH	$C_{22}H_{17}ClN_4O_2$	404.85	185	0.46	Ethanol/DMSO	76%

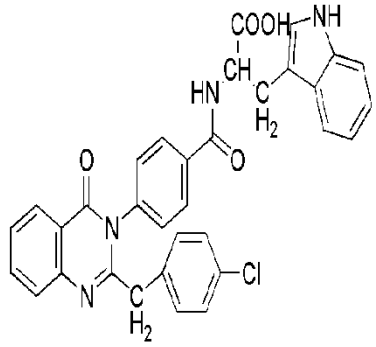
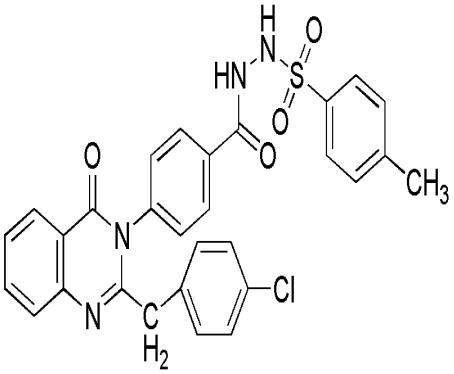
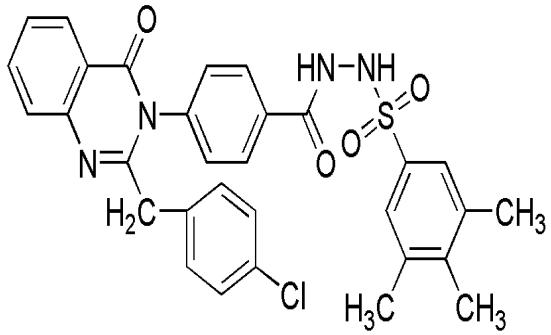
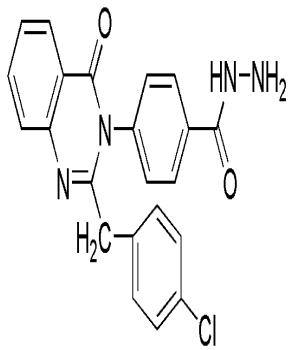
SPECTRAL STUDIES

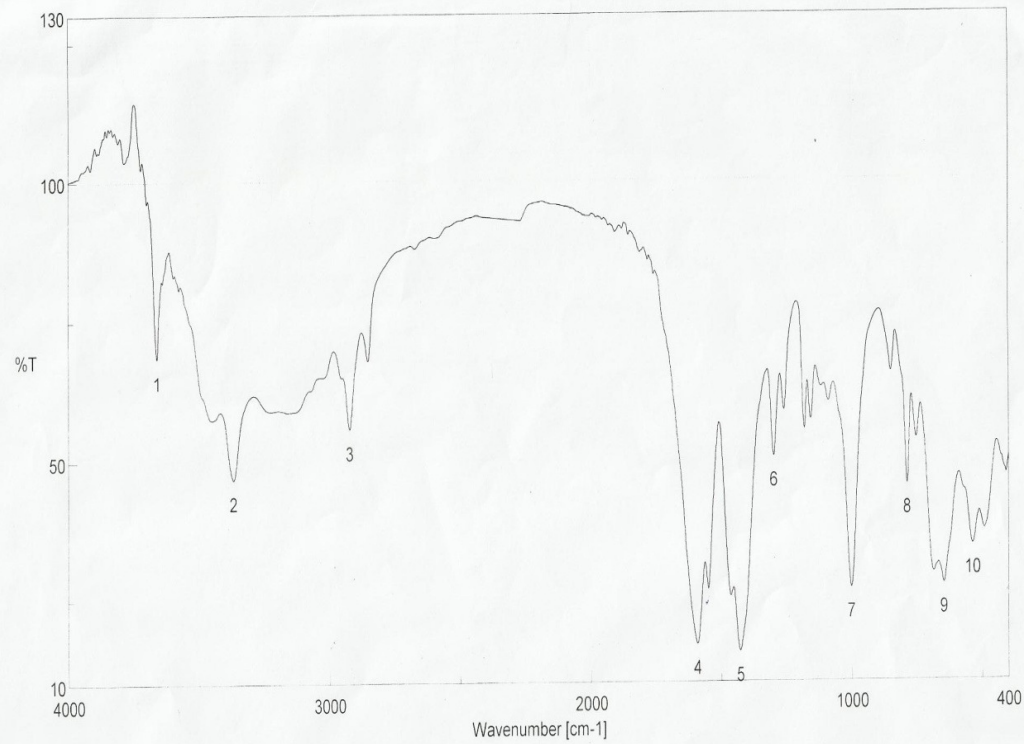
SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS[43]

Code	Structure	IR(cm^{-1})	NMR(δ ppm)	MASS(m/z, amu)

C-GY	 (4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzamido)acetic acid	2923.03(COOH Stretching) 1675.05(C=O Stretching) 1592.91(C=N Stretching) 1299.79(C-N Stretching) 789.70(Chlorine group Stretching)	7.4 – 7.9 (d, 4H, ArH) 8.0 (t, 1H,NH) 3.7 (S, 2H,CH_2) 10.7(S, 1H, COOH)	M/Z(446.98) Base peak(142.97)
C-GU		3127.15(COOH Stretching) 1675.05(C=O Stretching) 1592.91(C=N Stretching) 1200.20(C-N Stretching) 780.07(Chlorine group Stretching)		
C-PA	 (4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzamido)-3-phenylpropanoic acid	3142.15(COOH Stretching) 1616.20(C=C Stretching in aromatic) 1592.91(C=N Stretching) 780.07(Chlorine group Stretching) 697.15(MonoSubstituted benzene)		

C-TY	 (4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzamido)-3-(4-hydroxyphenyl)propanoic acid	3565.15(OH group Stretching) 3142.15(COOH Stretching) 1596.91(C=N Stretching) 827.17(P-substituted benzene)		
C-CY	 (4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzamido)-3-mercaptopropanoic acid	3142.95(COOH Stretching) 1550.25(C=N Stretching) 1515.17(C-S Stretching) 1300.20 (C-N Stretching) 788.20(Chlorine gp Stretching).		
C-HS	 (4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzamido)-3-(1H-imidazol-4-yl)propanoic acid	3127.95(COOH Stretching) 1671.05(C=O Stretching) 1581.25(C=N Stretching) 1438.85(C-H Stretching) 1200.20 (C-N Stretching) 789.20(Chlorine gp Stretching)		

C-TP	 (4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzamido)-3-(1H-indol-3-yl)propanoic acid	3123.15(COOH Stretching) 1661.37(C=O Stretching) 1548(C=N Stretching) 815.42(Chlorine gp Stretching)		
C-PT	 4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)-N'-tosylbenzohydrazide	2923.56(CH_3 Stretching) 1593(C=N Stretching) 1159.01(SO_2 Stretching) 788.74(Chlorine gp Stretching)	7.4-7.9(d,4H, ArH) 7.6(S,1H, NH) 2.0(S, 1H,CH_3) 0.9(S, 1H, CH_2)	M/Z(559.73) Base peak(110)
C-T		2925 (CH_3 Stretching) 1503.24(C=N Stretching) 1401.03 (C-Cl Stretching) 1302.08(S=O Streching) 1164.79(C-N Stretching)	7.3-7.9(M, 4H, ArH) 2.1(S, 1H, CH_2) 2.2-2.6(S, 3H,CH_3)	
C-HH	 4-(2-(4-chlorobenzyl)-4-oxoquinazolin-3(4H)-yl)benzohydrazide	3338.12(NH_2 Stretching) 1503.24(C=N Stretching) 1164.79(C-N Stretching) 790.03 (C-Cl Stretching)		

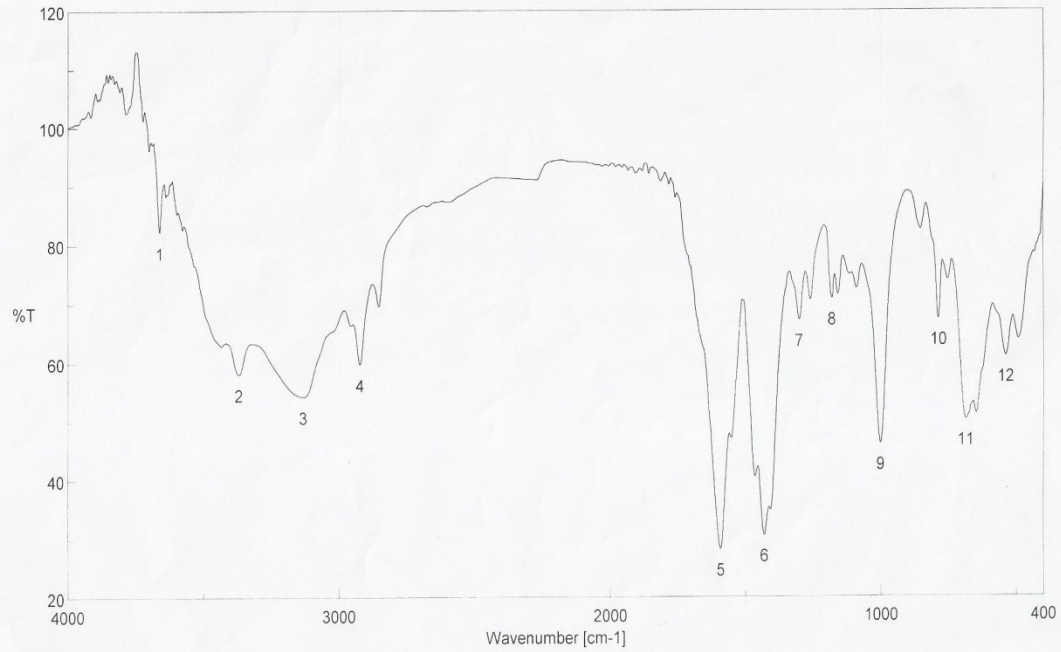


[Comment]

Sample Name	C-GY
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3660.23	68.4644	2	3368.07	46.729	3	2923.56	55.6162	4	1592.91	16.7114
5	1428.03	15.3876	6	1299.79	50.3598	7	1001.84	26.6422	8	789.707	45.2286
9	647.965	27.2738	10	537.078	34.2896						

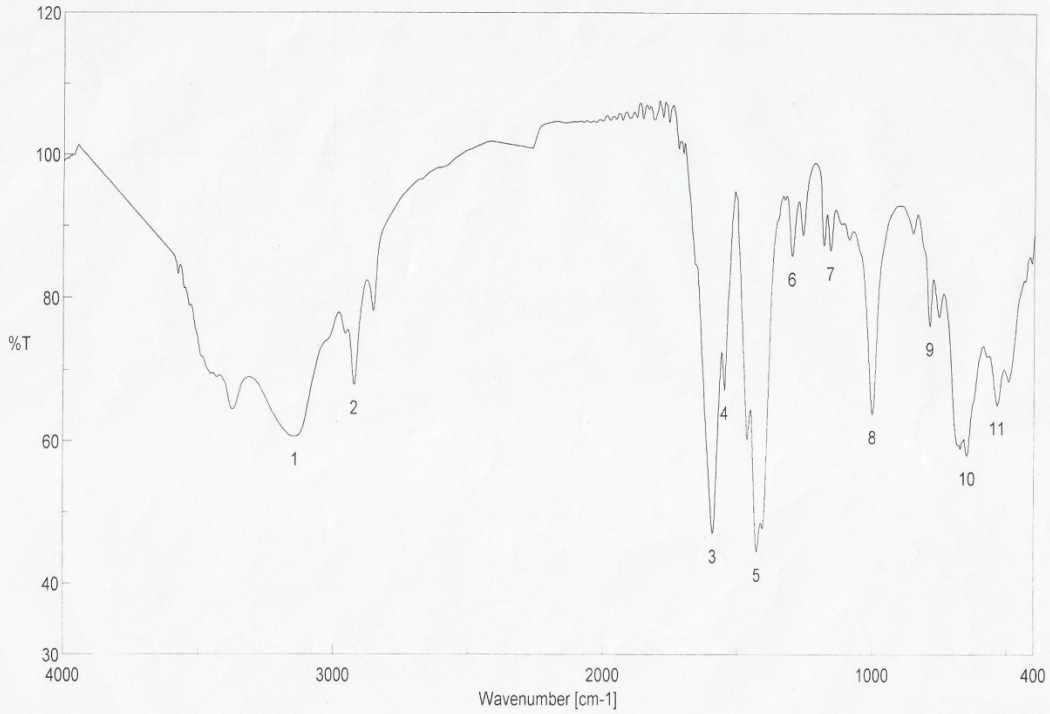


[Comment]

Sample Name	C-GU
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3660.23	82.1726	2	3369.03	58.0551	3	3132.79	54.1745	4	2922.59	59.6861
5	1592.91	28.1035	6	1429.96	30.4819	7	1301.72	67.1578	8	1182.15	70.7909
9	1001.84	46.0665	10	787.779	67.3397	11	686.534	50.2136	12	538.042	60.9943

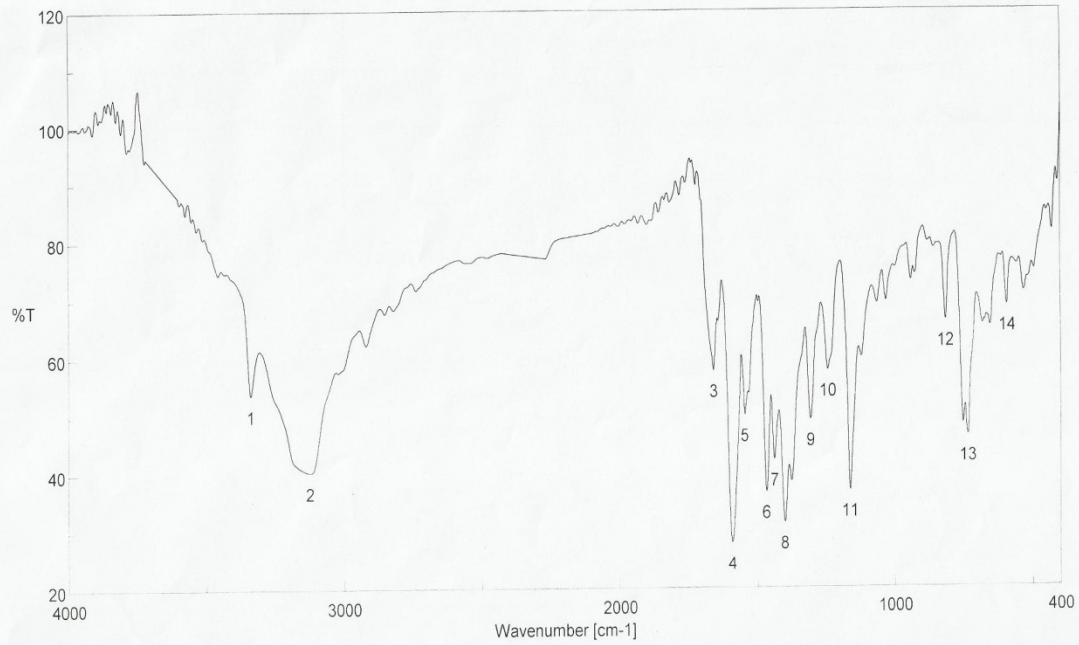


[Comment]

Sample Name	C-PA
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3142.44	60.5882	2	2922.59	67.8533	3	1592.91	47.0032	4	1550.49	67.1054
5	1428.03	44.3893	6	1300.75	85.8052	7	1159.01	86.5974	8	1000.87	63.7657
9	788.743	76.1026	10	648.929	57.9877	11	536.114	65.0655			

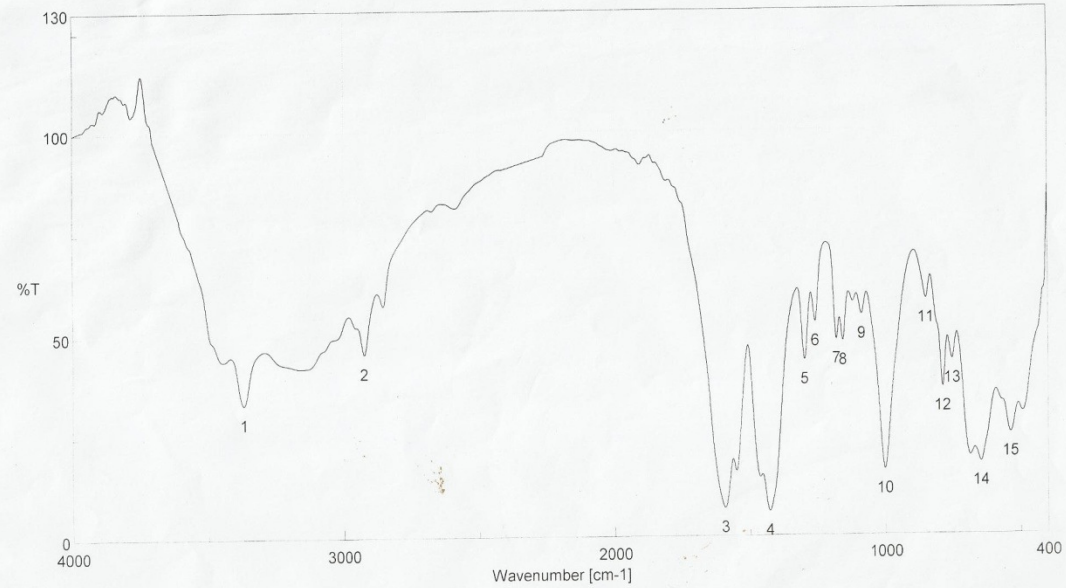


[Comment]

Sample Name	C-TP
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3338.18	53.675	2	3123.15	40.2989	3	1661.37	57.6344	4	1592.91	27.7802
5	1548.56	49.9279	6	1468.53	36.5548	7	1439.6	42.1673	8	1402	31.2542
9	1308.46	49.0668	10	1245.79	57.6241	11	1164.79	36.7549	12	815.742	66.152
13	735.71	46.2334	14	593.004	68.7166						

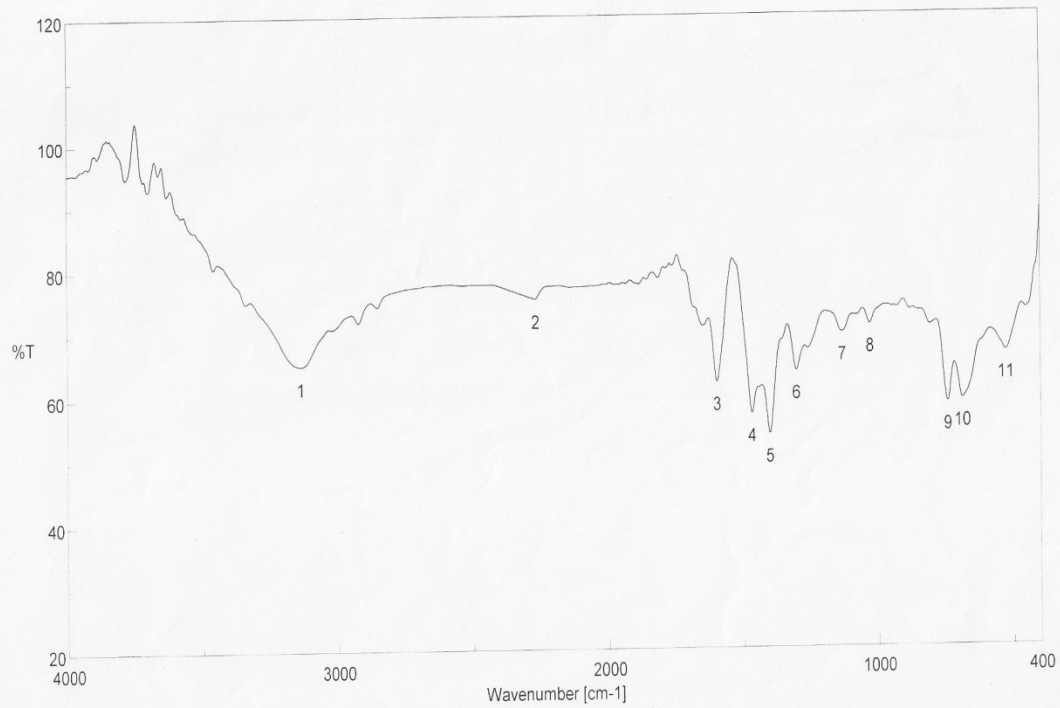


[Comment]

Sample Name	C-PT
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3368.07	33.0568	2	2923.56	45.3645	3	1593.88	6.89186	4	1428.99	6.04274
5	1299.79	43.3473	6	1260.25	52.745	7	1183.11	48.3656	8	1159.01	47.9653
9	1089.58	54.4291	10	1001.84	16.1089	11	850.454	58.2139	12	788.743	36.373
13	754.031	43.2242	14	647.965	17.8333	15	538.042	24.9997			

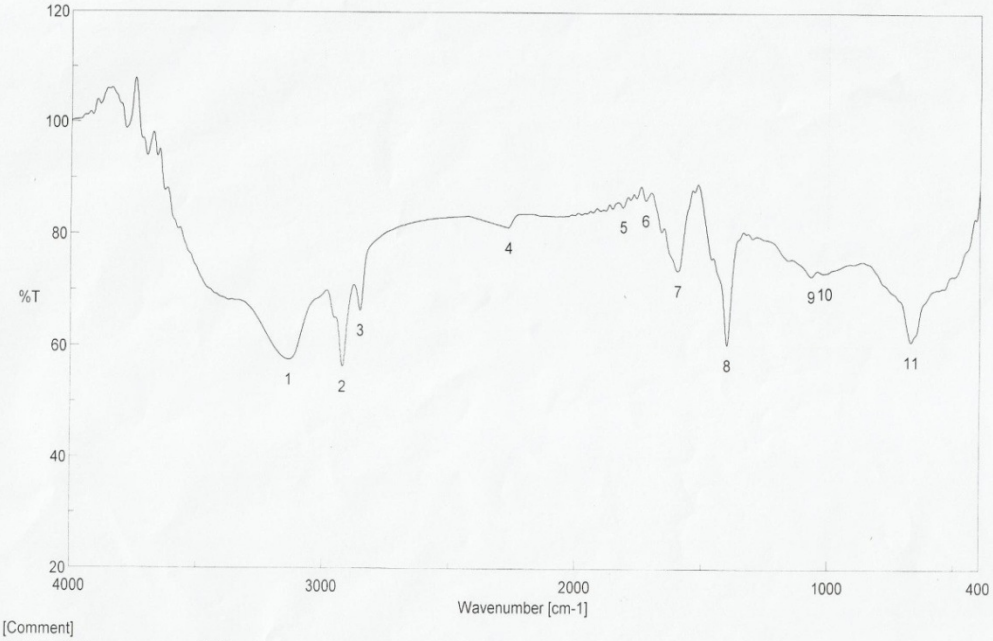


[Comment]

Sample Name	C-T
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3137.62	65.0932	2	2266.91	75.2553	3	1598.7	61.9916	4	1468.53	56.9921
5	1402	53.7216	6	1303.64	63.6372	7	1133.94	69.6088	8	1029.8	70.8329
9	743.424	58.4612	10	689.427	58.9716	11	527.436	66.4558			

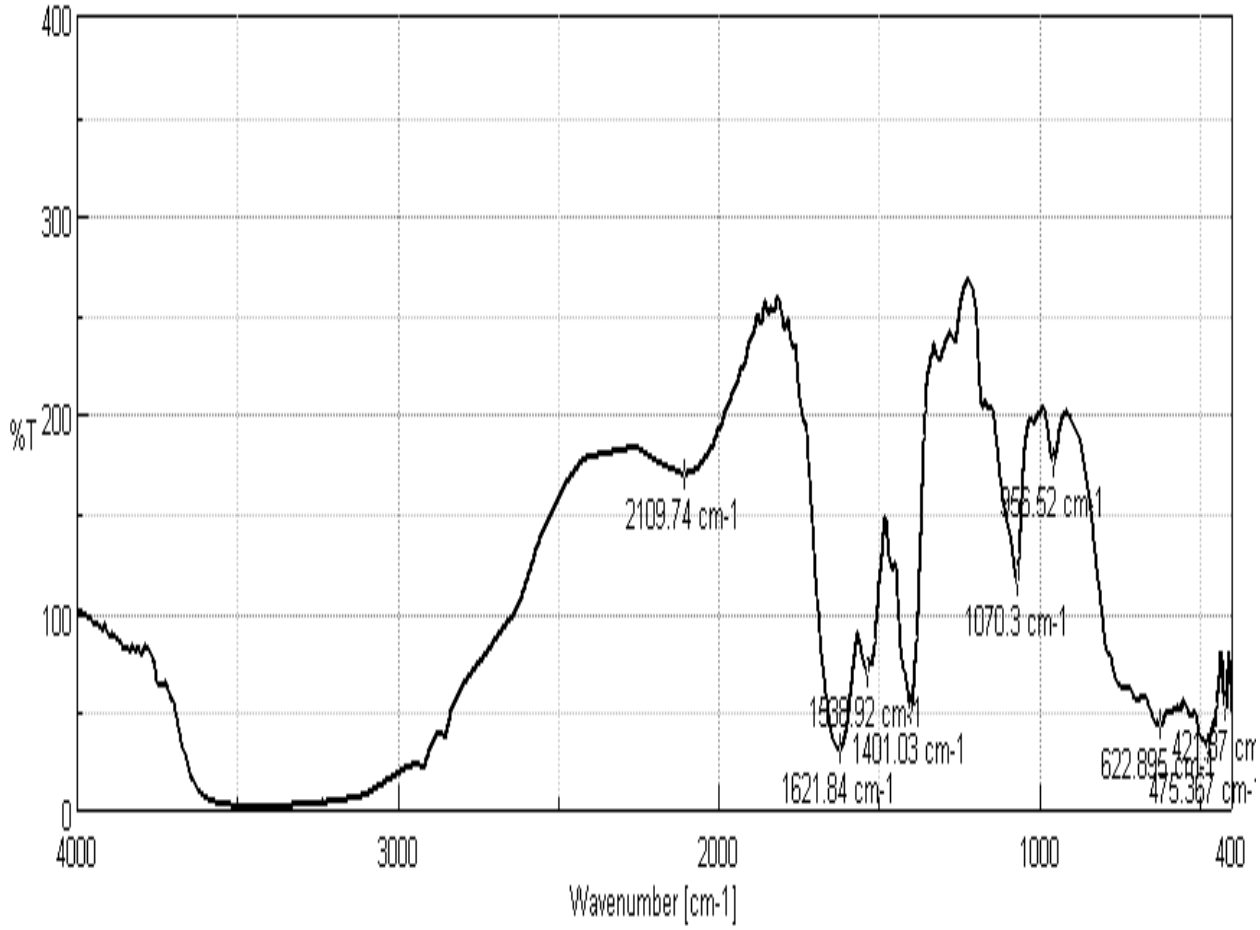


[Comment]

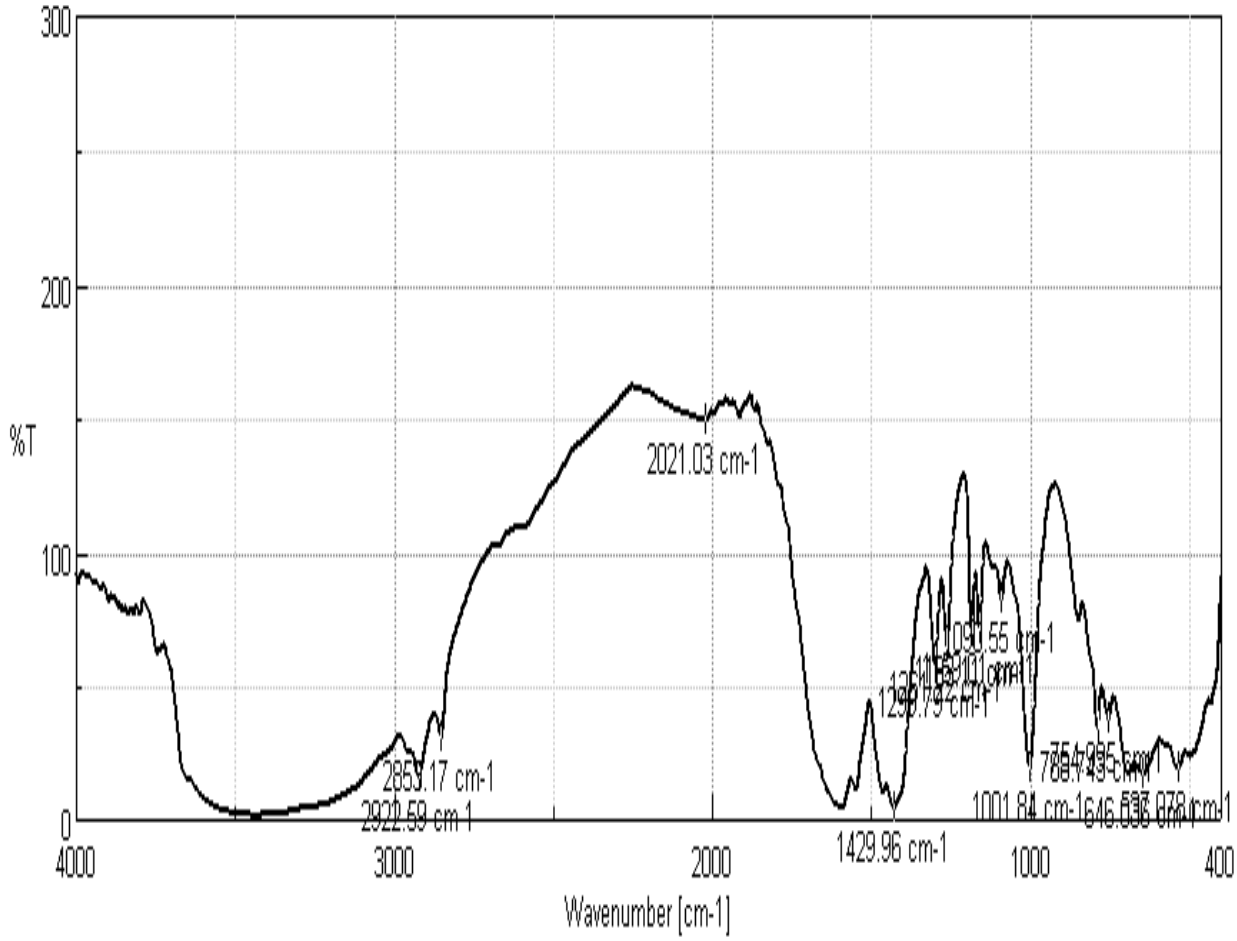
Sample Name	C-TY
Comment	
User	
Division	
Company	KMCH

[Result of Peak Picking]

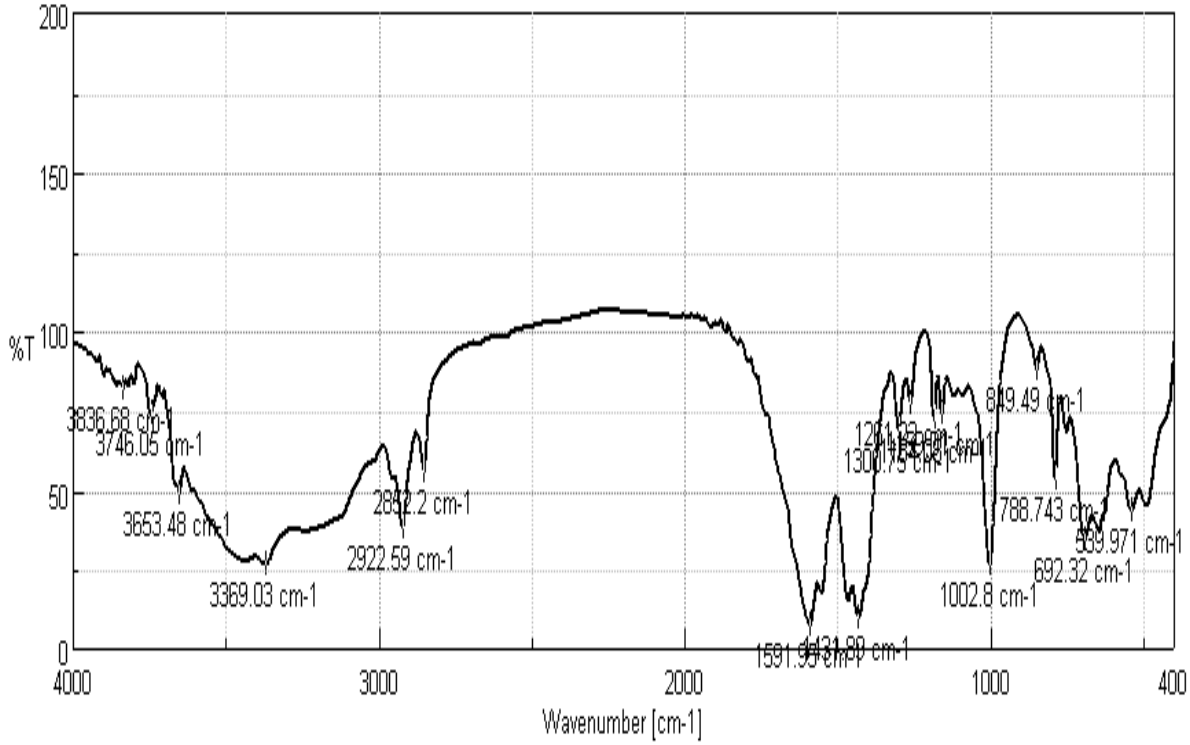
No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3136.65	57.4959	2	2922.59	56.274	3	2853.17	66.3595	4	2266.91	81.1829
5	1811.79	85.0194	6	1724.05	86.1563	7	1596.77	73.6107	8	1402	60.3079
9	1069.33	72.7054	10	1014.37	73.207	11	672.071	60.9771			


400
300
200
100
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
2109.74 cm-1
1621.84 cm-1
1401.03 cm-1
1070.3 cm-1
622.895 cm-1

C-HH

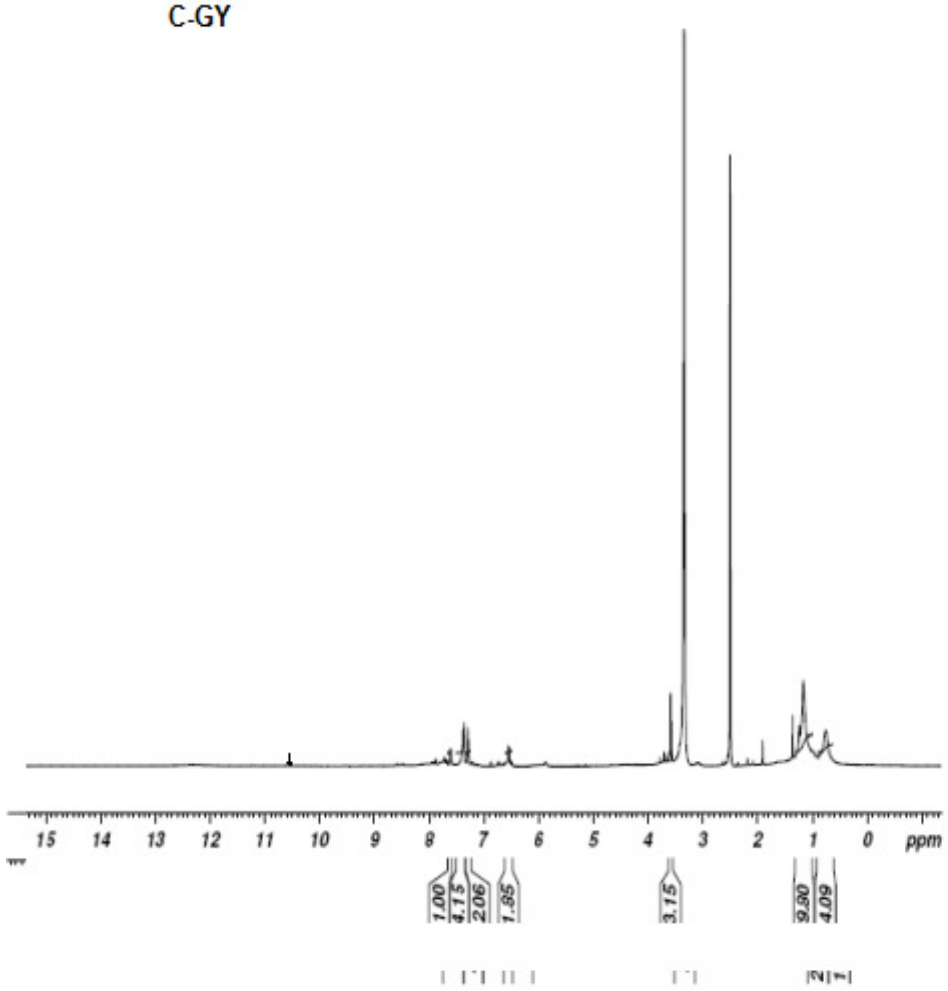

300
200
100
0
%T
2021.03 cm-1
2853.17 cm-1
2922.59 cm-1
1429.96 cm-1
1001.84 cm-1
4000
3000
2000
1000
400
Wavenumber [cm-1]

C-CY


200
150
100
50
0
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]
3746.05 cm-1
3653.48 cm-1
3369.03 cm-1
2922.59 cm-1
1591.95 cm-1
1002.8 cm-1
788.743 cm-1
692.32 cm-1

C-HS

C-GY



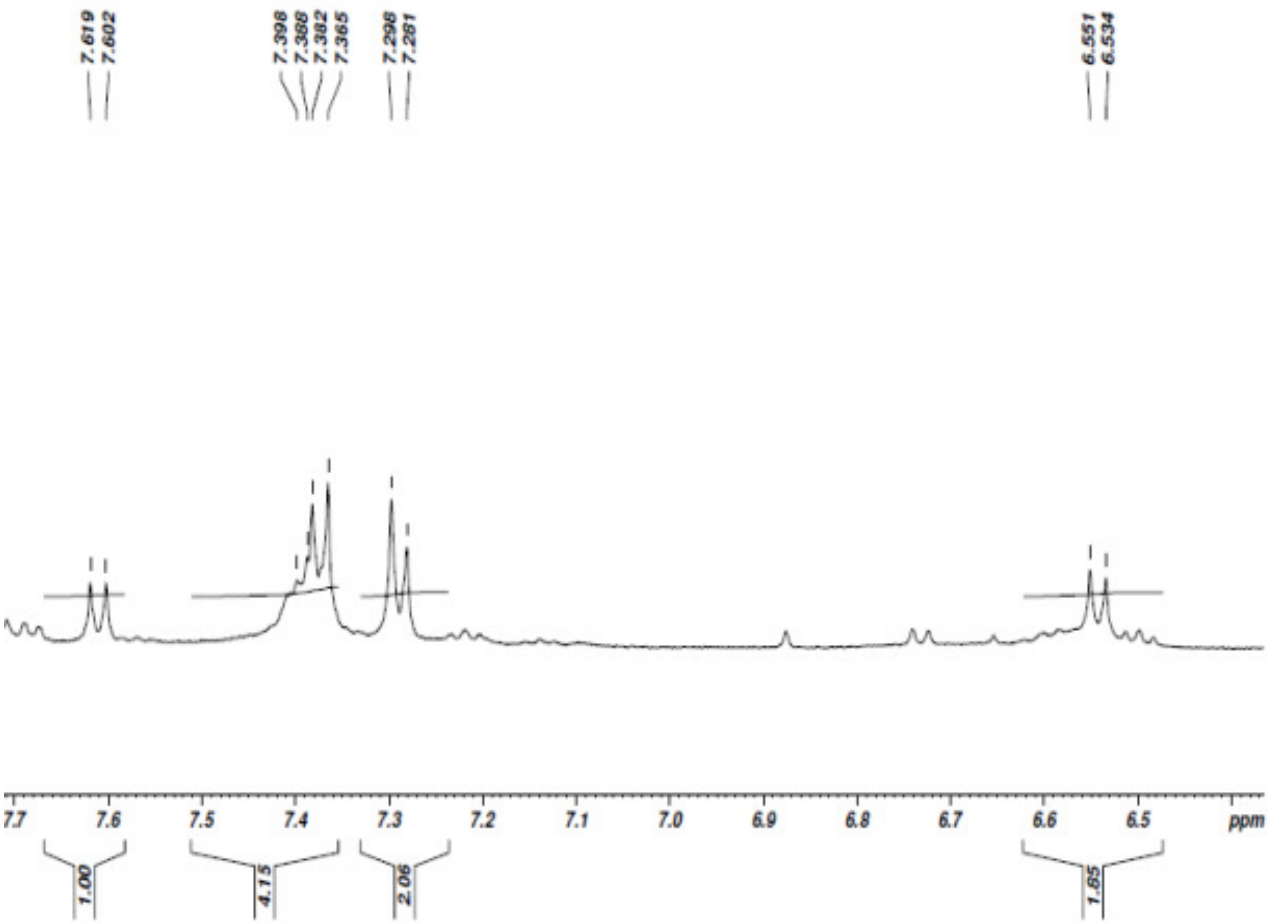

```
Current Data Parameters
NAME          Nov23-2011
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20111123
Time               10.26
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                32768
SOLVENT            DMSO
NS                    32
DS                     2
SWH           10330.578 Hz
FIDRES        0.315264 Hz
AQ            1.5860212 sec
RG                   203
DW                48.400 use
DE                 6.50 use
TE                296.8 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.65 use
PL1                0.00 dB
PL1W        23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
SI                32768
SF          500.1300000 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00
```

C-GY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7.388
7.382
7.365
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7.281
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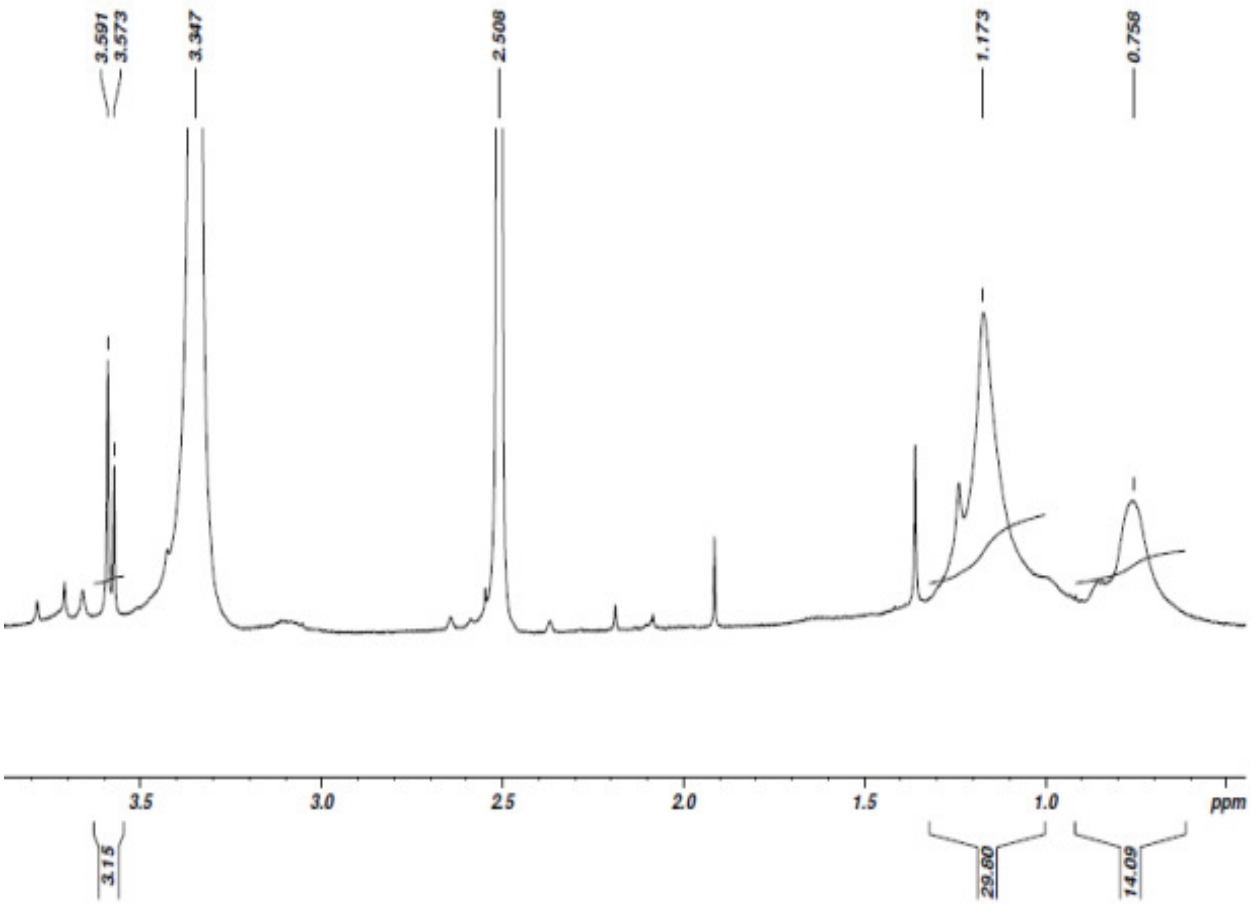
6.534
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
ppm
1.00
4.15
2.06
1.85

C-GY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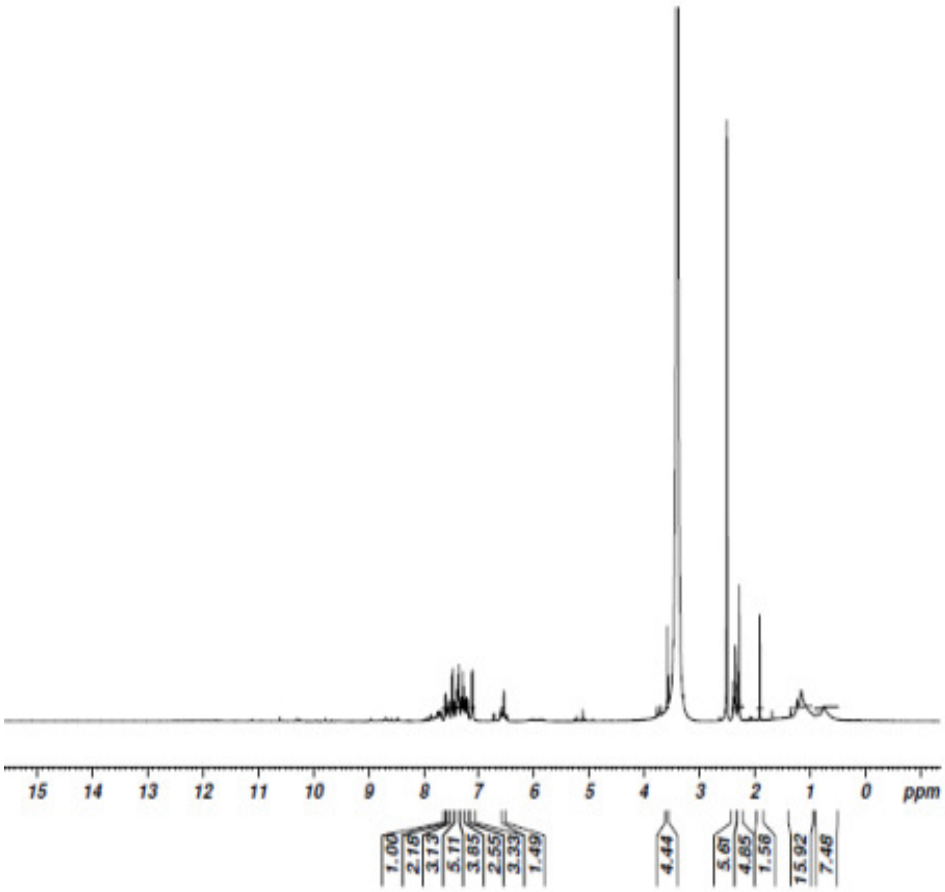
2.508
1.173
0.756
3.5
3.0
2.5
2.0
1.5
1.0
ppm
3.15
29.60
14.09

C-PT

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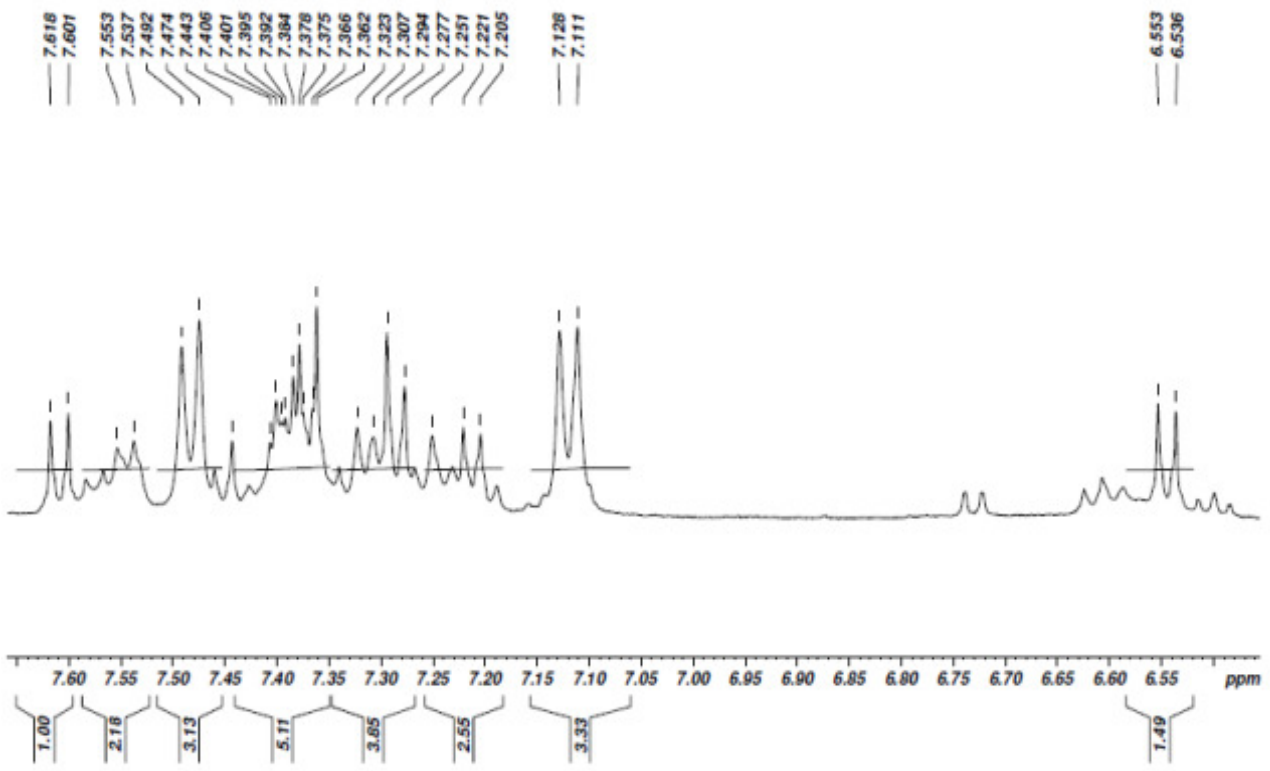
Current Data Parameters
NAME                  Nov23-2011
EXPNO                          8
PROCNO                         1

F2 - Acquisition Parameters
Date_                   20111123
Time                       10.17
INSTRUM                    spect
PROBHD     5 mm PABBO BB-
PULPROG                     zg30
TD                         32768
SOLVENT                     DMSO
NS                            32
DS                             2
SWH                    10330.578 Hz
FIDRES                 0.315264 Hz
AQ                     1.5860212 sec
RG                           101
DW                        48.400 usec
DE                         6.50 usec
TE                       296.8 K
D1                     1.00000000 sec
TD0                            1

======== CHANNEL f1 ========
NUC1                          1H
P1                        10.65 usec
PL1                        0.00 dB
PLW                   23.53637505 W
SFO1                  500.1330885 MHz

F2 - Processing parameters
SI                         32768
SF                    500.1300000 MHz
WDW                           EM
SSB                            0
LB                         0.30 Hz
GB                             0
PC                          1.00
```

C-PT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7.443
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7.395
7.392
7.384
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7.375
7.366
7.362
7.323
7.307
7.294
7.277
7.251
7.221
7.205
7.128
7.111
6.553
6.536
7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10 7.05 7.00 6.95 6.90 6.85 6.80 6.75 6.70 6.65 6.60 6.55 ppm
1.00
2.16
3.13
5.11
3.65
2.55
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1.49

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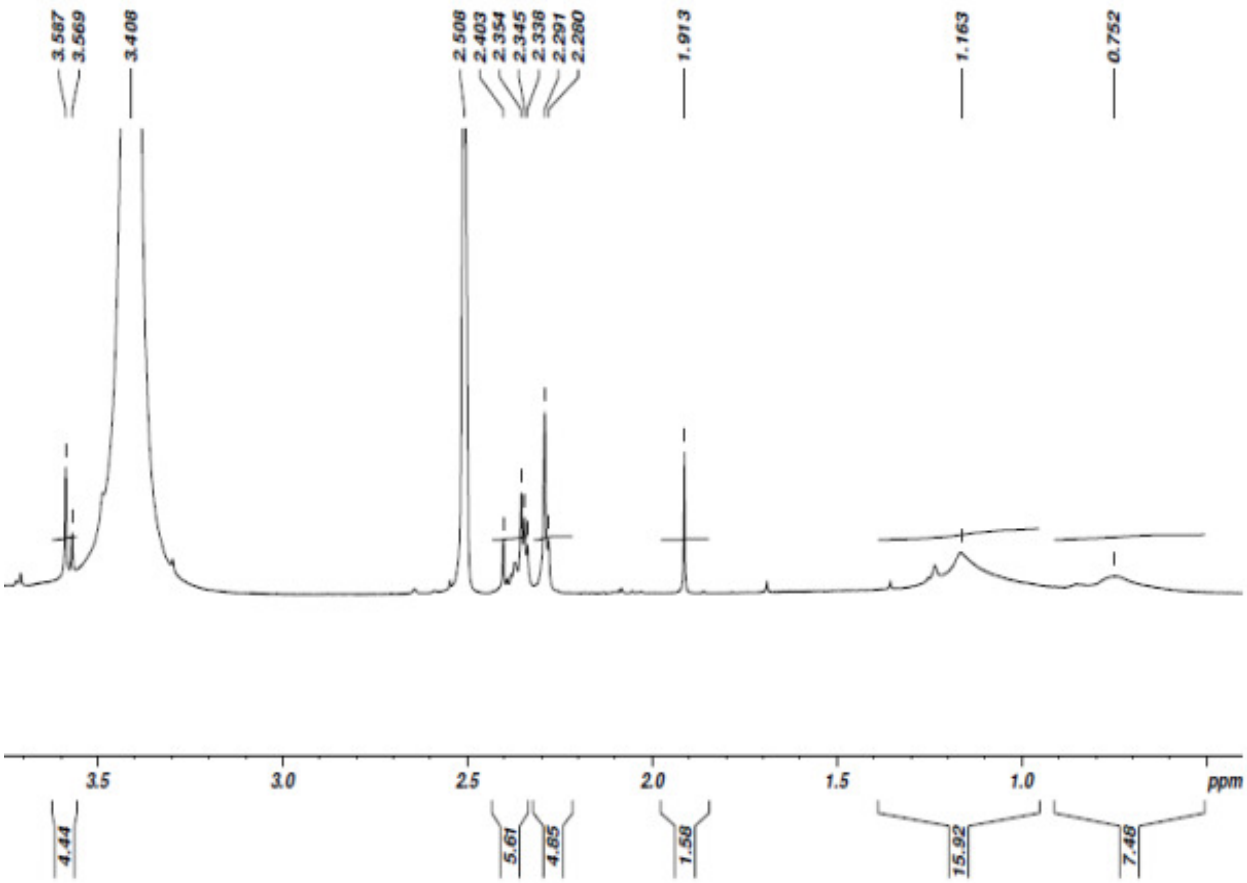
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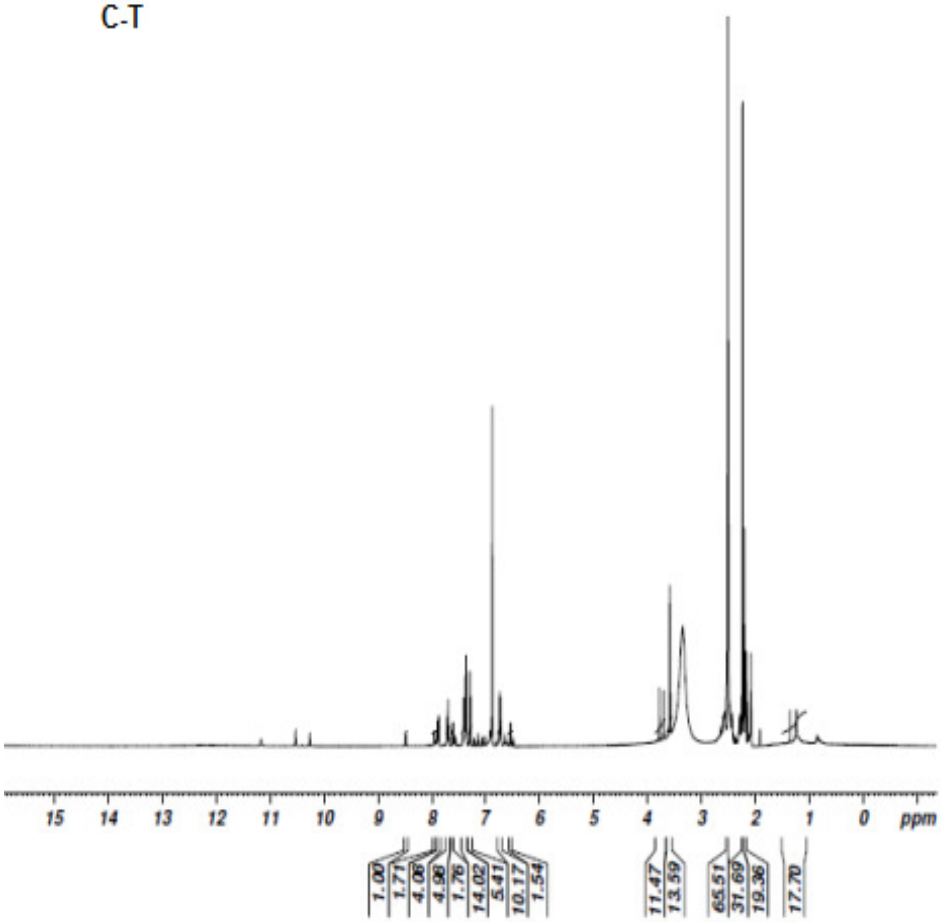


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TE               296.0 K
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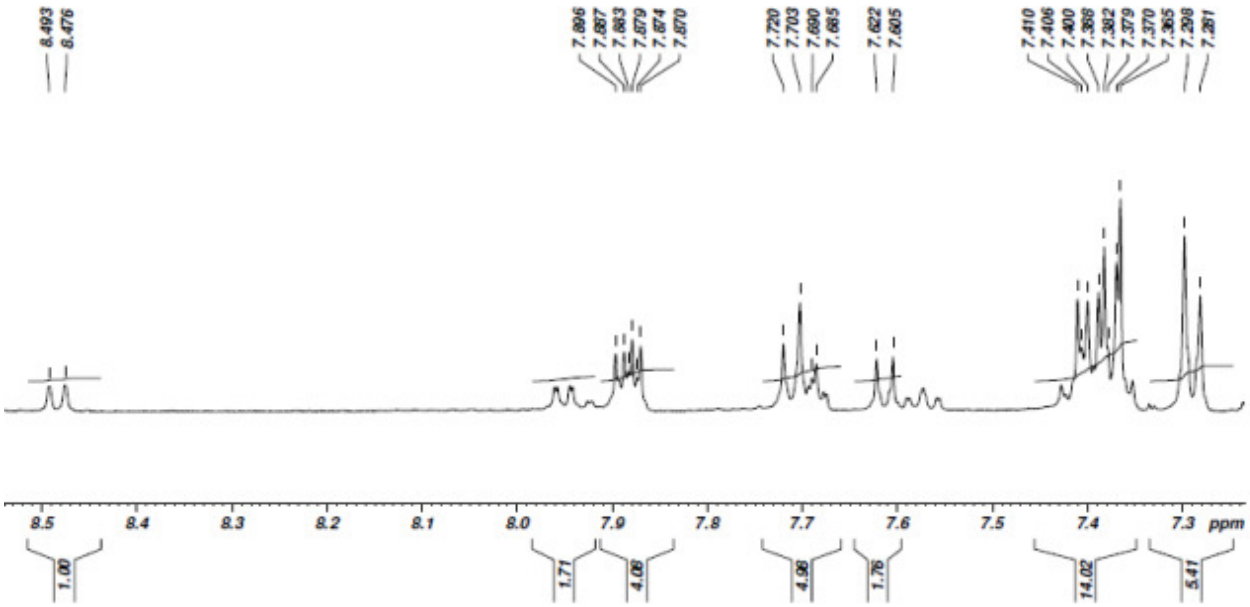
C-T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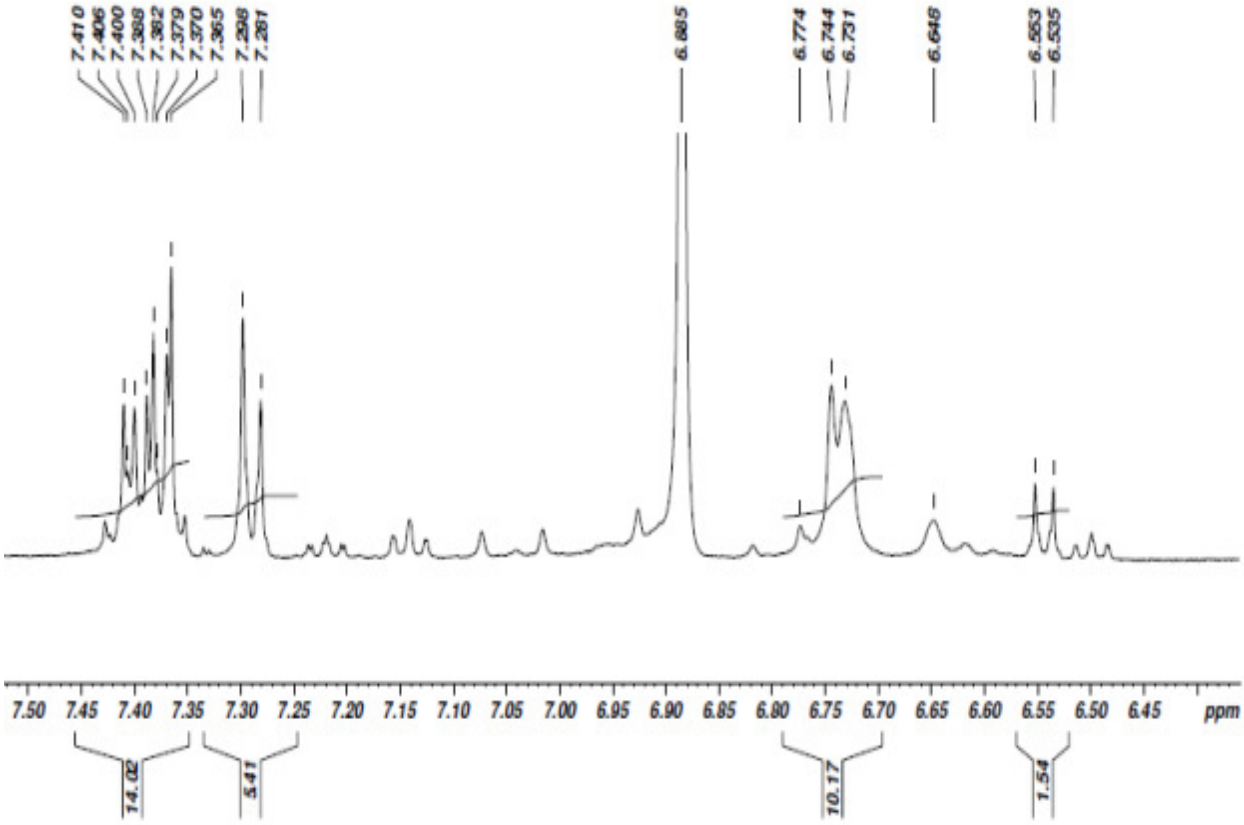
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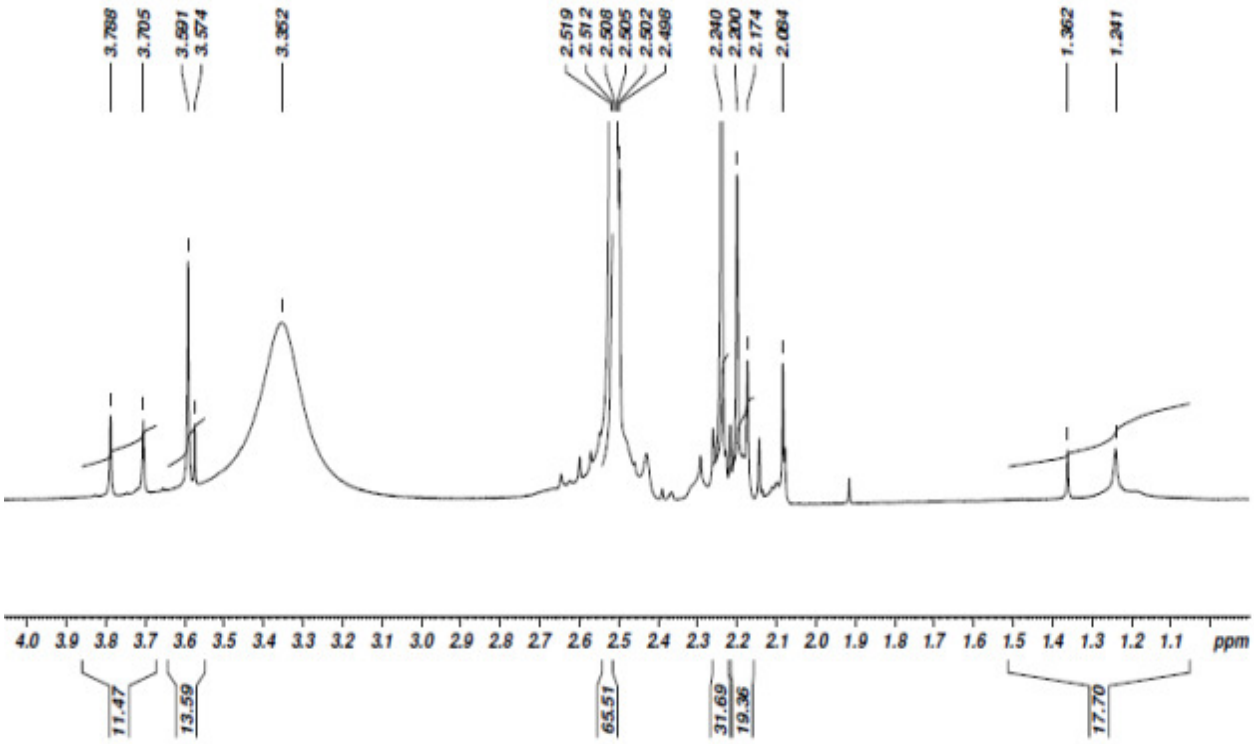
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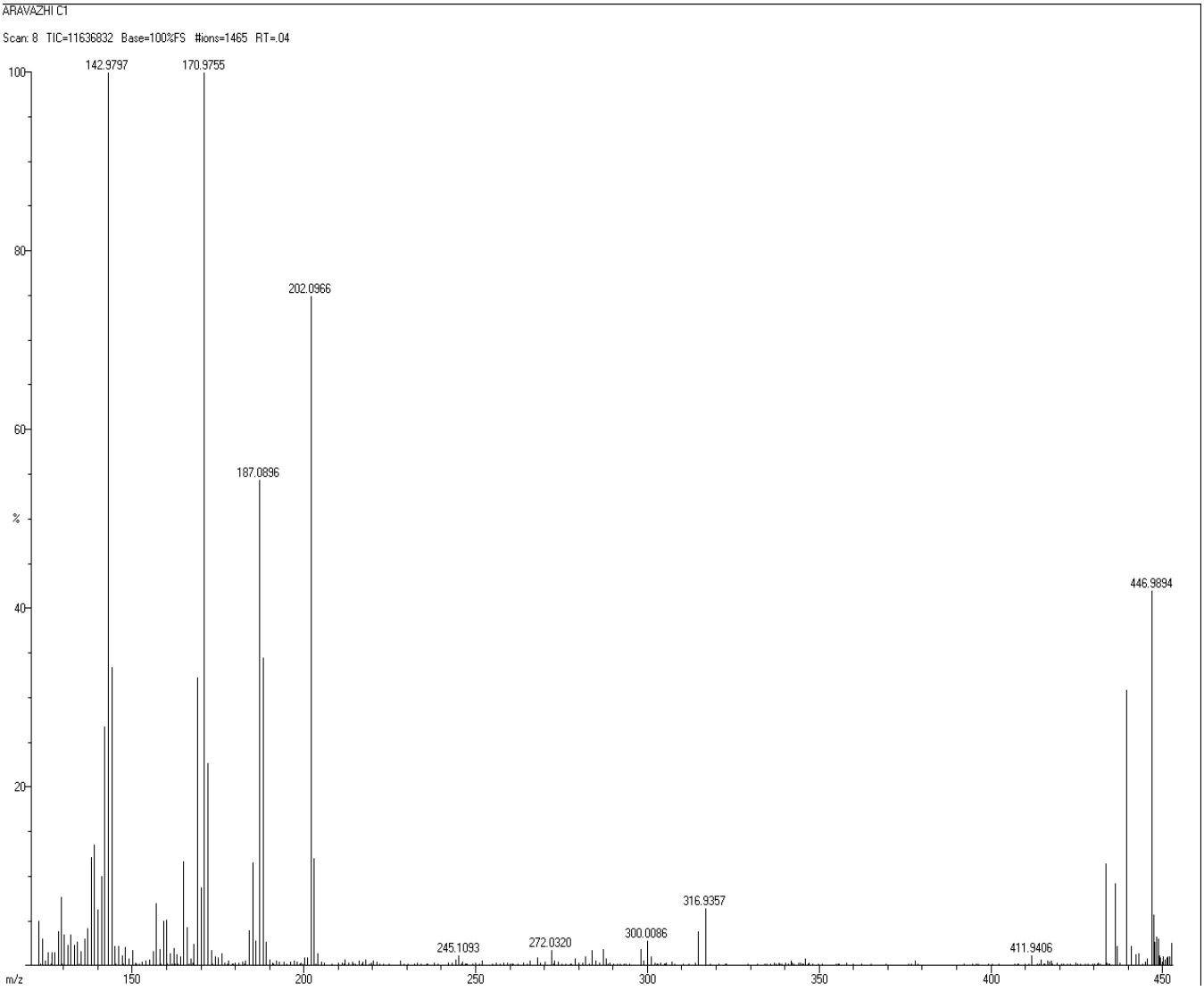
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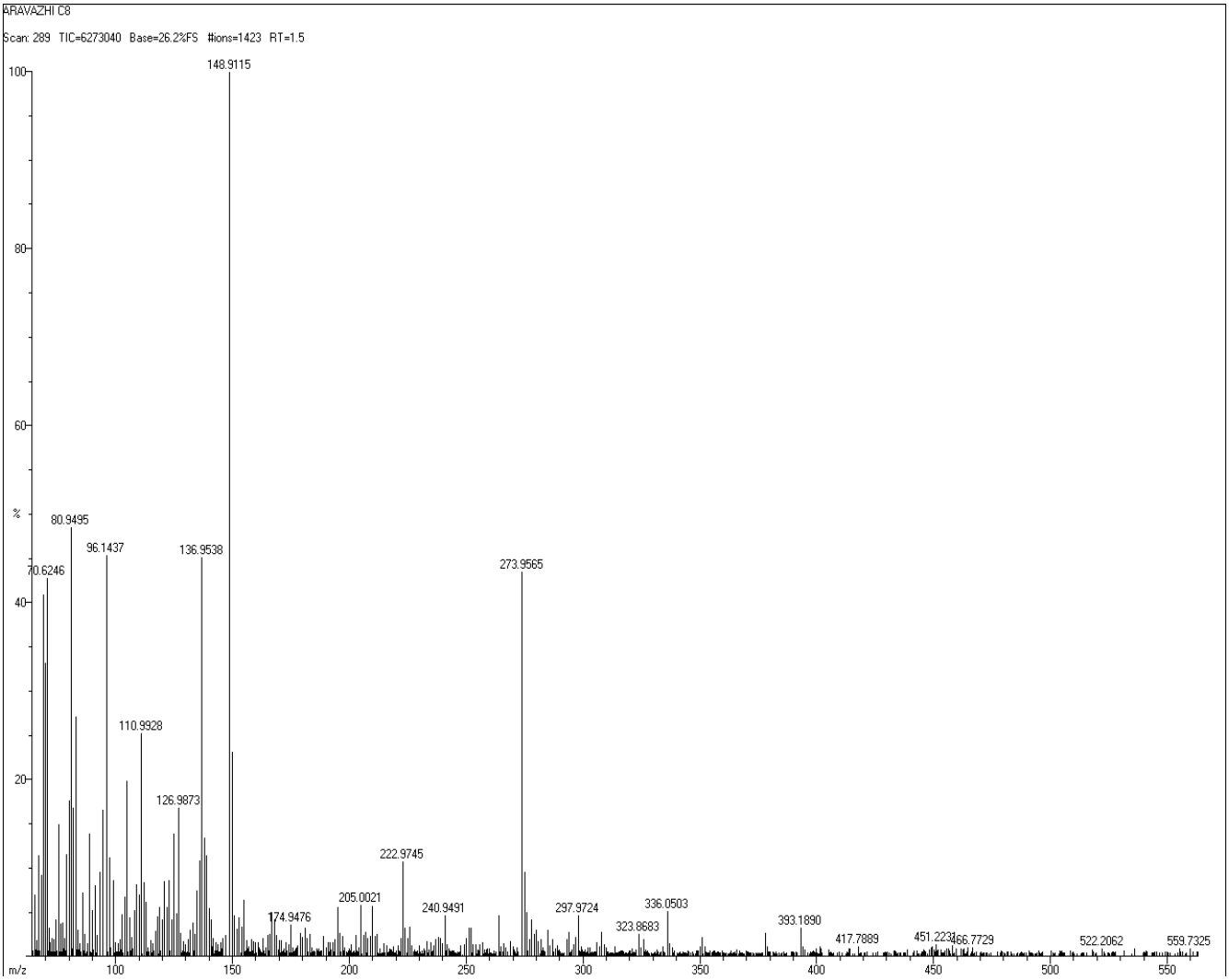
C-GY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%
m/z

C-PT

Chapter 5

Biological Screening

INVITRO ANTI-OXIDANT SCREENING OF SYNTHESIZED COMPOUNDS

- In-vitro Anti-oxidant screening of synthesized compounds were done by using three methods

  - ✓ DPPH method
  - ✓ FRAP method and

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical-scavenging activity:

Procedure:[39]

The free radical-scavenging activity of the synthesized compounds were measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1–5 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0$$

where A_0 was the absorbance of the control (blank, without compounds) and A_t was the Absorbance in the presence of the compounds. All the tests were performed in triplicate and the graph was plotted with the mean values.

Ferric reducing antioxidant power (FRAP) assay:[39]

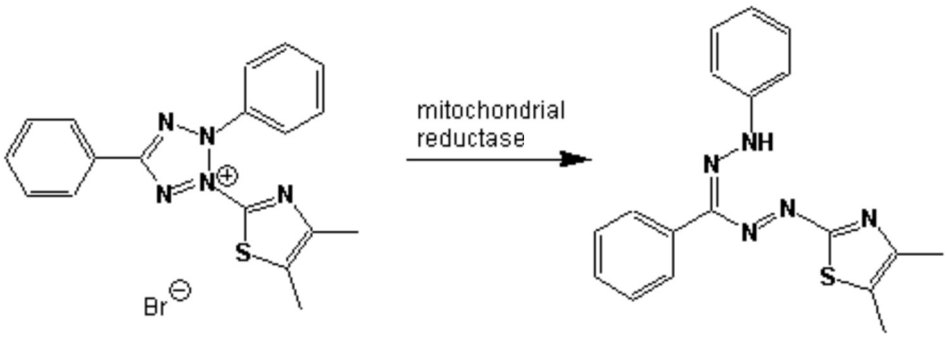
FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)- s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content (Benzie and Strain, 1996). 0.2 ml of the compound is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3$. 6H2O solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox can be used as a positive control.

IN VITRO ANTICANCER SCREENING OF SYNTHESIZED COMPOUNDS BY MTT ASSAY METHOD

MTT ASSAY

PRINCIPLE

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole), is reduced to purple formazan by mitochondrial succinate dehydrogenase in living cells.The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The insoluble purple formazan product is converted to a colored solution using a solubilization agent (usually either DMSO, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid). The absorbance of this colored solution is then quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. Since reduction of MTT can only occur in metabolically active cells, this assay gives a direct measure of the viability of cells.



The human cervical cancer cell line (**HeLa)** was obtained from National Centre for cell Science (NCCS), Pune. The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS).

For screening experiment,[40] the cells were seeded into 96-well platesin 100µl of medium containg 5% FBS, at plating density of 10,000 cells/well and incubated at 37^0C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of samples. The samples were solubilised in Di methylsulfoxide and diluted in serum free medium. After 24h , 100µl of the medium containg the samples at various concentration (eg; 6.25, 12.5, 25, 50mM etc…..) was added and incubated at 37^0C, 5% CO2, 95% air and 100% relative humidity for 48h. Triplicate was maintained and the medium containing without extracts were served as control.

After 48h,15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. the medium with MTT was then flicked off and the formed formazen crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell inhibition = 100- Abs (sample)/Abs (control) x 100.

Non-linear regression graph was plotted between % cell inhibition and Log10 concentration and IC50 was determined using Graph Pad Prism Software.

ANTI-BACTERIAL SCREENING OF THE SYNTHESIZED COMPOUNDS BY DISC DIFFUSION METHOD

PROCEDURE:

Preparation of Muller Hinton Agar Medium:

Composition of Muller Hinton Agar Medium

SL.NO	INGREDIENTS	QUANTITY
1	Beef extract	10g
2	Casein acid hydrolysate	17.5g
3	Starch	1.5g
4	Agar	20 g
5	Distilled water	1000ml

Specified amount of Muller Hinton agar was taken along with 1000ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120°C for 15 minutes. The sterile medium was poured into the sterile Petri dish and allowed to solidify.

Preparation of plates:

Muller Hinton agar medium was prepared and transferred into sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized bacterial inoculums of *Vibrio cholera, Escherchia coli, Bacillus subtilis, Bacillus linctus, Micrococcus luteus, Staphylococcus aureus, Klebsiella pneumonia, Coryne bacterium* were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs (10µg /disc) in dimethyl sulphoxide and standard ciprofloxacin

5µg disc were placed on the inoculated agar medium. All petriplates were incubated at 37˚C for 24 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured. The antibacterial activity was evaluated by measuring zone of inhibition in mm.

Determination of Minimum Inhibitory Concentration of Synthesized Compounds (MIC) by Serial dilution method:

- The serial dilution of known concentration of compound solution was made from the stock (100 mg/ml) by using Muller Hinton broth using the method described below.
- The tubes were labeled 1 to 8 and 1 ml of Muller Hinton broth were added to the first 5 tubes and 8th tube, then added 0.5 ml Muller Hinton broth to 6th and 7th tubes.
- One ml of different synthesized compounds was added to the 1st tube, mixed and transfer 1ml serially up to tube 5.From the 5th tube transfer 1ml to 6th tube. Mixed and transfer 0.5 ml to the 7th tube. Each tube, 1 to 7 contains 1ml diluted extract.
- The 8th tube was the control.
- With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 37^{0} c for 24hrs.
- The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

ANTI-FUNGAL SCREENING OF THE SYNTHESIZED COMPOUNDS BY DISC DIFFUSION METHOD

PROCEDURE

Preparation of Sabourands dextrose broth: [41'42]

Composition of Sabourands dextrose broth

SL.NO	INGREDIENTS	QUANTITY
1	Dextrose	40g
2	peptone	10g
3	water	1000ml

Specified amount of dextrose and peptone was taken along with 1000ml of distilled water in a conical flask and heated in a steam bath to dissolve. The pH was maintained at 7.6 ± 0.2 and sterilized in an autoclave at 15 lb pressure, 120°C for 15 minutes. The sterile medium was poured into the sterile Petri dish and allowed to solidify.

Preparation of plates:

Sabourands dextrose broth medium was prepared and transferred into sterile Petri plates aseptically (thickness of 5-6mm). The plates were allowed to dry at room temp. The plates were inverted to prevent condensate falling on the agar surface. The layers of the medium are uniform in thickness, is done by placing the plates on a leveled surface. Standardized fungal inoculums of *Aspergillus niger, Aspergillus fumigatus, Aspergillus parasiticus, Candida albicans,* were applied to the plates and spreaded uniformly over the surface of medium by using a sterile Non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs (10µg /disc) in dimethyl sulphoxide and standard Clotrimazole 10µg/disc were placed on the inoculated agar medium. All petriplates were incubated at 27˚C -28˚C for 48 hrs. After the incubation diameter of zone of inhibition produced by the sample were measured.

Determination of Minimum Inhibitory Concentration for Synthesized Compounds (MIC) by Serial dilution method:

- The serial dilutions of known concentration of compound solution are made from the stock (100 mg/ml) by using Sabourands dextrose broth using the method described below.

- The tubes were labeled 1 to 8 and 1 ml of Sabourands dextrose broth were added to the first 5 tubes and 8th tube, then added 0.5ml of Sabourands dextrose broth to 6th and 7th tubes.
- One ml of different synthesized compounds was added to the 1st tube, mixed and transfer 1ml serially up to tube 5.From the 5th tube transfer 1ml to 6th tube. Mixed and transfer 0.5 ml to the 7th tube. Each tube, 1 to 7 contains 1ml diluted extract.
- The 8th tube was the control.
- With a standardized micro pipette, add a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 26-28 ^{0} c for 48hrs.
- The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC.

Chapter 6

Results and Discussion

ANTI OXIDANT SCREENING

Anti Oxidant activity of synthesized compounds

INVITRO ANTIOXIDANT

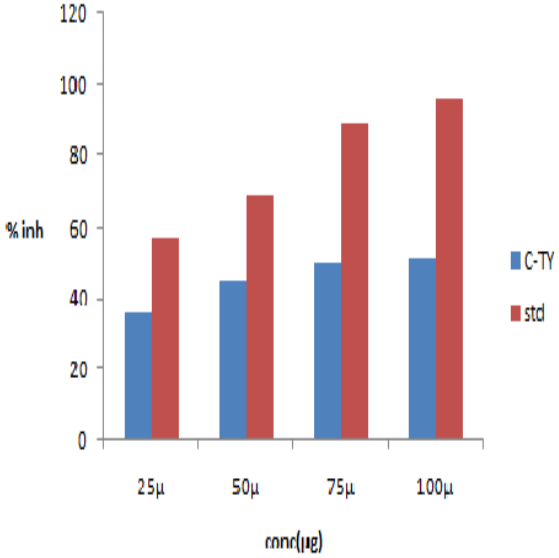
DPPH RADICAL SCAVENING ACTIVITY:

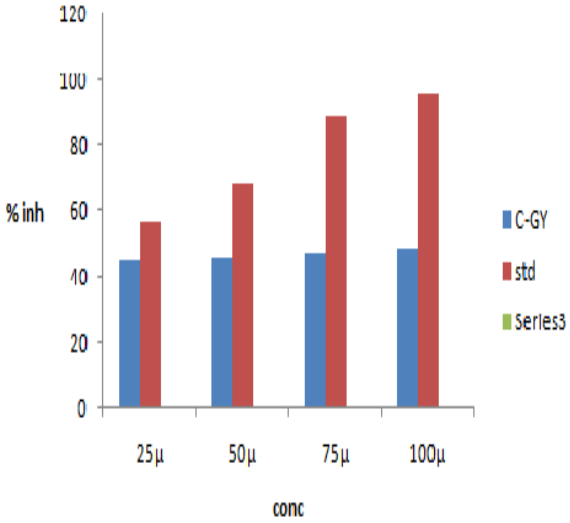
DPPH Radical scavenging (antioxidant) activity was determined by the method modified by *Hatano* et al.,(1989).

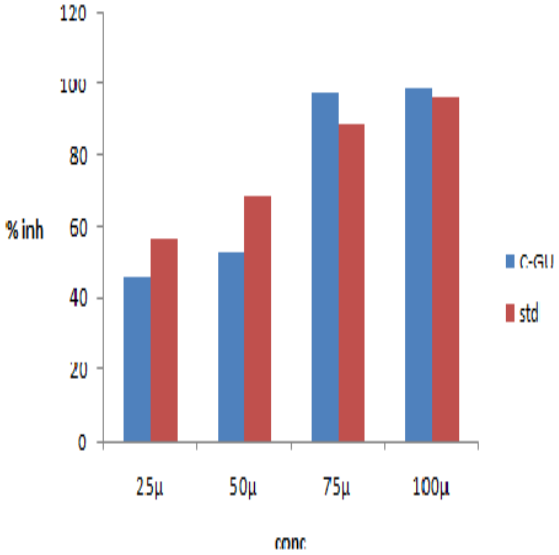
Percentage of Inhibition

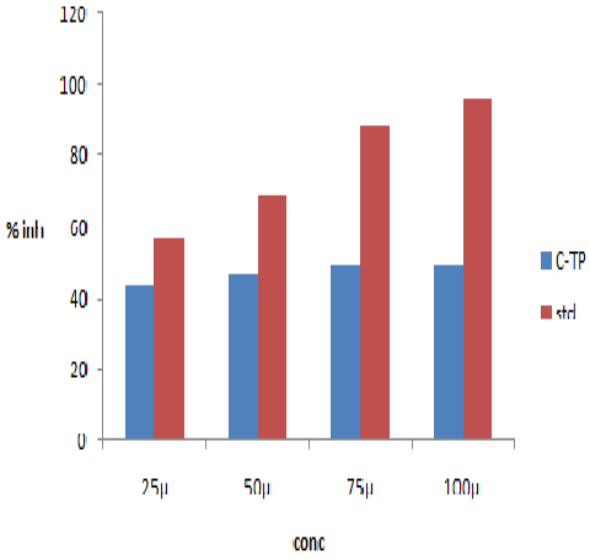
Concentration	C-TY	C-GY	C-GU	C-CY	C-HS	C-PT	C-T	C-HH	C-TP	C-PA	Ascorbic Acid
25µ	35.59	44.72	45.17	29.36	16.96	45.92	72.24	47.52	43.76	45.40	56.7
50µ	44.49	45.71	53.00	43.40	37.09	49.41	53.52	46.59	46.56	41.97	68.9
75µ	49.69	47.46	97.12	78.71	78.70	44.60	65.11	67.14	48.87	49.51	89.0
100µ	50.51	48.37	98.36	45.56	45.56	46.76	93.34	63.58	49.07	46.58	96.0
EC_{50}	35	27	28	42	73	28	17	26	26	27	22

ANTIOXIDANT ACTIVITY OF COMPOUNDS C-TY,C-GY,C-GU,C-TP AND ASCORBIC ACID









ANTI OXIDANT SCREENING

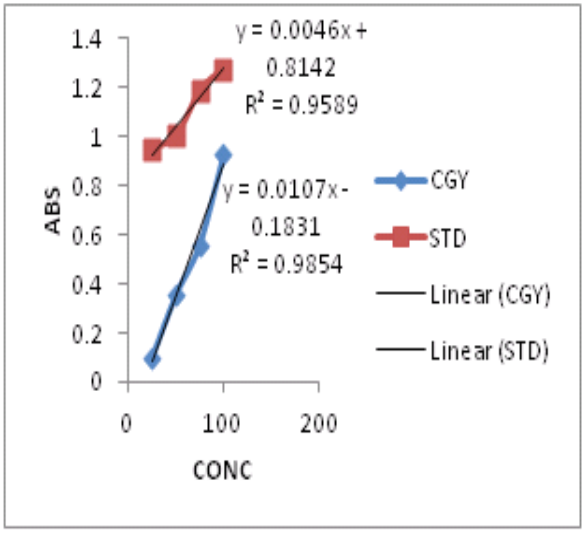
Anti Oxidant activity of synthesized compounds

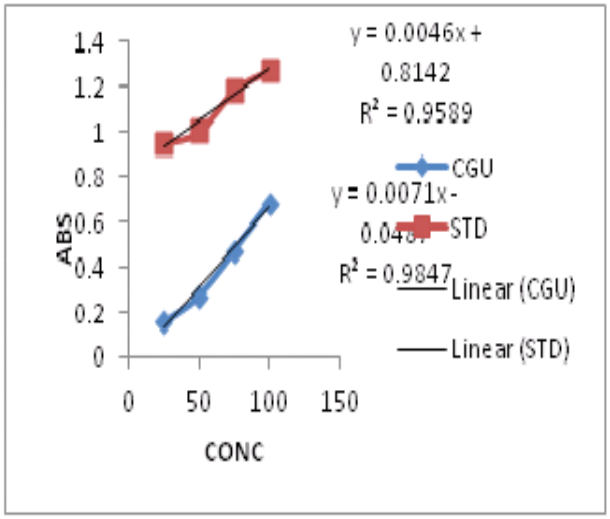
INVITRO ANTIOXIDANT

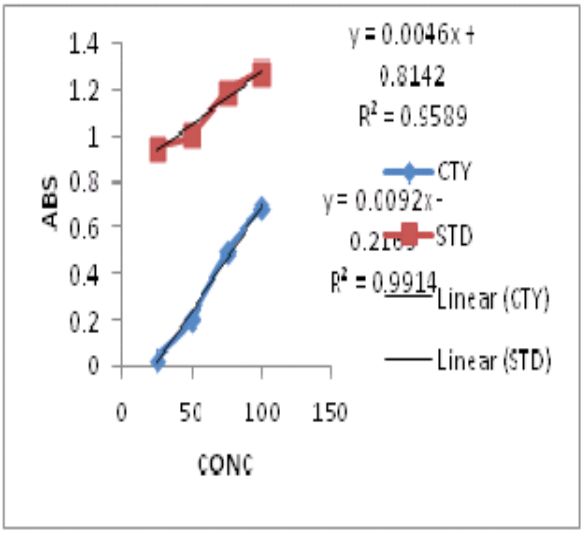
Ferric reducing antioxidant power (FRAP) assay:

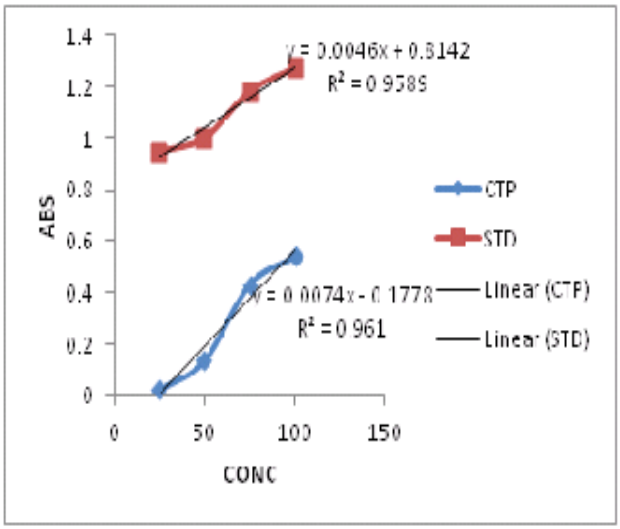
Absorbance											
Concn	C-TY	C-GY	C-GU	C-CY	C-T	C-HS	C-PT	C-PA	C-TP	C-HH	Ascorbic Acid
25µ	0.0274	0.1021	0.1568	0.3673	0.2653	0.8768	0.2665	0.4903	0.0038	0.2758	0.9504
50µ	0.2055	0.3565	0.2743	0.4057	0.5086	0.7270	0.4601	0.4124	0.1366	0.6385	1.0020
75µ	0.4974	0.5612	0.4733	0.5469	0.5358	0.8258	0.3990	0.2229	0.4328	0.3083	1.1843
100µ	0.6940	0.9269	0.6853	0.1526	1.1480	0.4065	0.1148	0.3968	0.5398	0.1767	1.2743
R^2	0.95	0.95	0.96	0.75	0.62	0.85	0.79	0.52	0.95	0.65	0.98

ANTIOXIDANT ACTIVITY OF COMPOUNDS C-TY,C-GY,C-GU,C-TP AND ASCORBIC ACID









In vitro **anticancer screening**

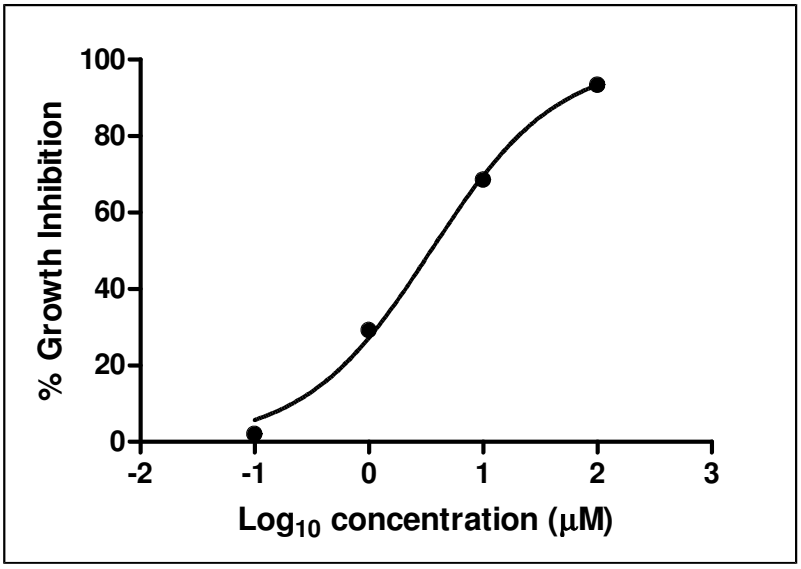
HeLa cell line inhibition by the synthesised compound(C-PT)

Conc(µm)	% cell Inhibition	conc	0.1(µm)	1(µm)	10(µm)	100(µm)	control
0.1	1.136364	ABS	0.396	0.394	0.381	0.357	0.415
1	1.948052		0.41	0.402	0.385	0.366	0.391
10	6.980514		0.412	0.412	0.38	0.377	0.426
100	10.71429	Avg	0.406	0.402	0.382	0.366	0.410

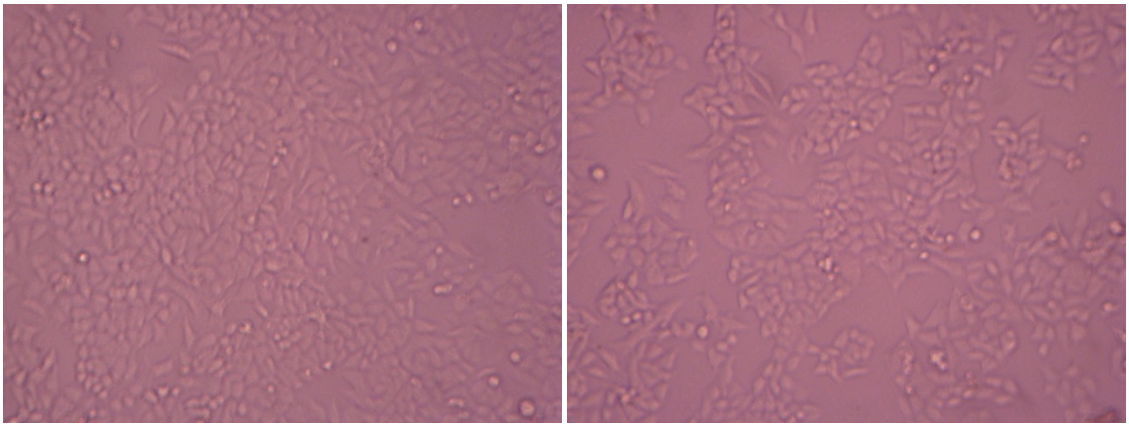
IC_{50} >100

HeLa cell line inhibition by the synthesised compound(C-GU)

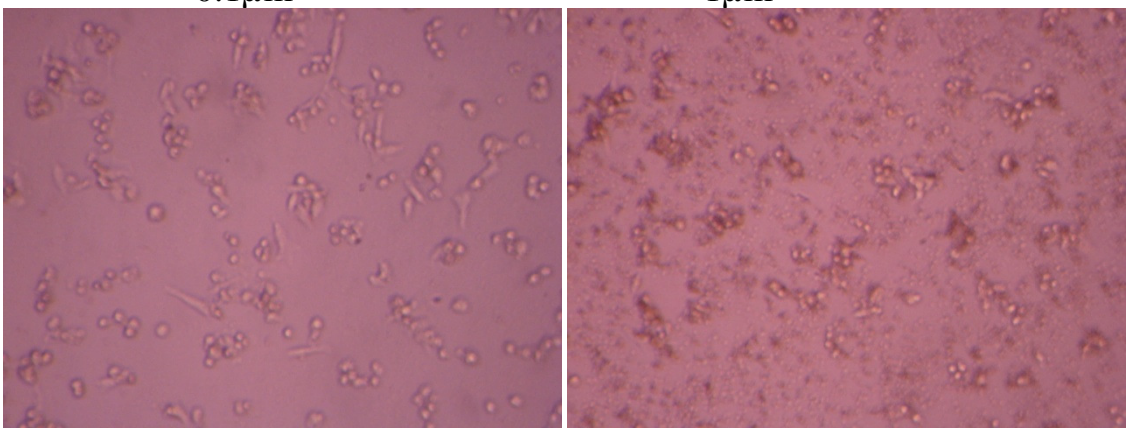
Conc(µm)	% cell Inhibition	conc	0.1(µm)	1(µm)	10(µm)	100(µm)	control
0.1	2.001334	ABS	0.469	0.355	0.158	0.023	0.476
1	29.21948		0.492	0.357	0.158	0.041	0.496
10	68.57905		0.508	0.349	0.155	0.035	0.527
100	93.39872	Avg	0.48927	0.5328	0.157	0.033	0.49967



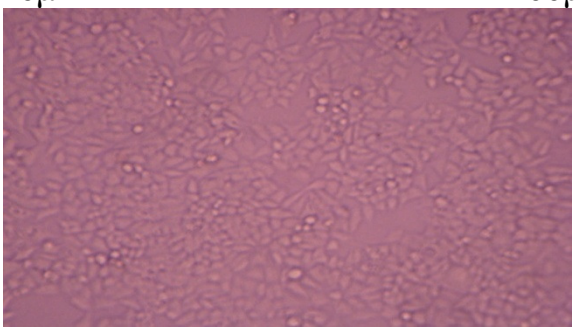
Photograph of HeLa cell line inhibition by the synthesised compound(C-GU)



0.1µm 1µm



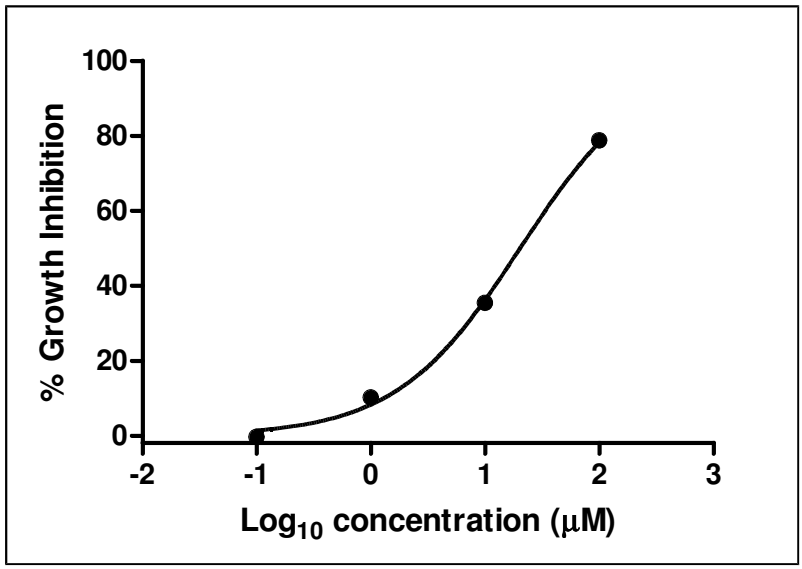
10µm 100µm



control

HeLa cell line inhibition by the synthesised compound(C-T)

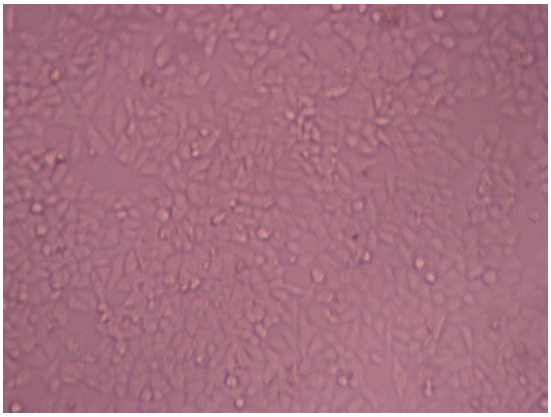
Conc(µm)	% cell Inhibition	conc	0.1(µm)	1(µm)	10(µm)	100(µm)	control
0.1	-0.40027	ABS	0.503	0.464	0.315	0.117	0.476
1	10.07338		0.498	0.445	0.323	0.103	0.496
10	35.3569		0.504	0.439	0.331	0.099	0.527
100	78.71915	Avg	0.501	0.449	0.323	0.106	0.499



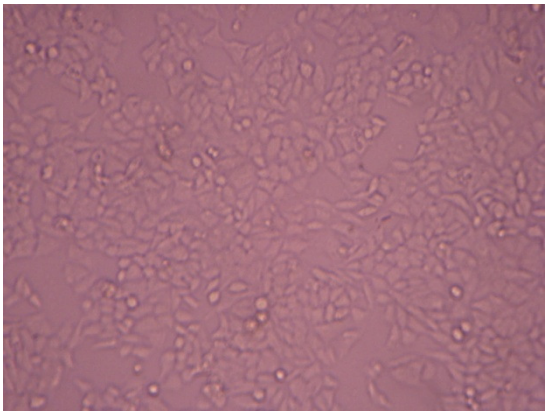
HeLa cell line inhibition by the synthesised compound(C-GY)

Conc(µm)	% cell Inhibition	conc	0.1(µm)	1(µm)	10(µm)	100(µm)	control
0.1	1.13632	ABS	0.4	0.372	0.359	0.353	0.415
1	7.95454		0.42	0.376	0.362	0.351	0.391
10	11.85065		0.398	0.386	0.365	0.35	0.426
100	14.44805	Avg	0.406	0.378	0.362	0.351	0.410

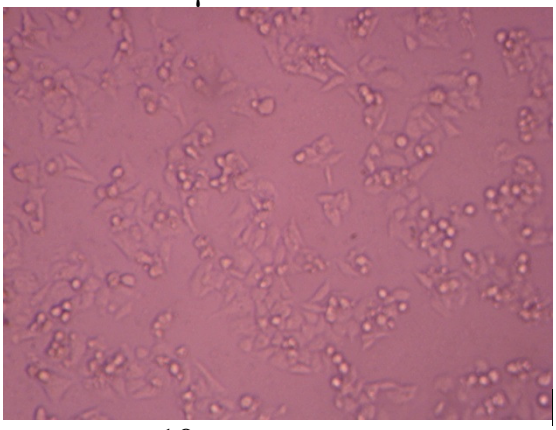
Photograph of HeLa cell line inhibition by the synthesised compound(C-T)



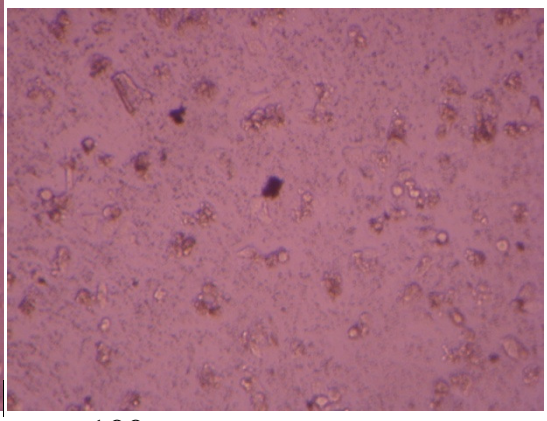
0.1µm



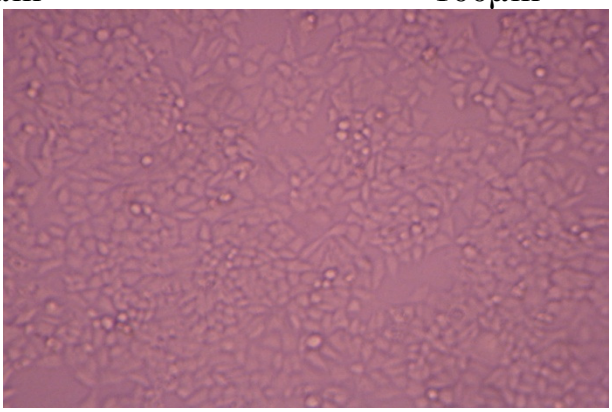
1µm



10µm



100µm



control

RESULT

Sr.no	Compound	Conc (µm)	%cell Inhibition	IC_{50}(µm)
1	C-GY	0.1	1.13632	–
		1	7.95454	
		10	11.85065	
		100	14.44805	
2	C-GU	0.1	2.001334	3.5(µm)
		1	29.21948	
		10	68.57905	
		100	100.9339	
3	C-PT	0.1	1.13634	–
		1	1.94805	
		10	6.980519	
		100	10.71429	
4	C-T	0.1	-0.40027	20.27(µm)
		1	10.07338	
		10	35.3569	
		100	78.71915	

Anti bacterial screening Disc diffusion method :

S. No	Micro Organism	Zone of Inhibition
		Compound (10µg/disc)

		C-GY	C-GU	C-PA	C-TY	C-CY	C-HS	C-TP	C-PT	C-T	C-HH	STD Ciprofloxacin (10µg/disc)
1.	*M.luteus*	12	-	-	3	3	10	-	-	12	15	16
2.	*S.aureus*	13	6	5	4	2	6	14	7	14	16	17
3.	*B.subtilis*	10	4	5	-	3	7	5	4	6	11	17
4.	*C.diphtheriae*	9	3	3	-	3	11	-	-	4	12	13
5.	*E.coli*	4	-	11	3	4	10	5	3	6	14	17
6.	*P.aureginosa*	11	-	3	4	-	3	5	4	7	15	16
7.	*R.rubrum*	-	3	-	11	-	11	4	3	3	13	15
8.	*V.cholerae*		-	13	3	3	13	-	-	3	16	17

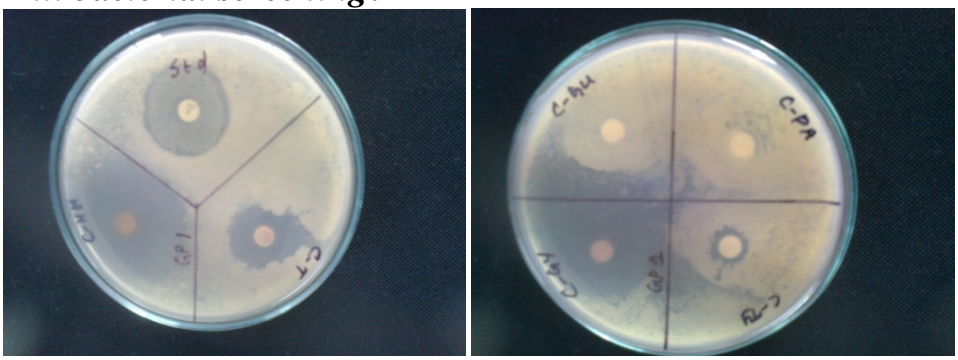
SERIAL DILUTION METHOD

MIC values of the synthesized compounds

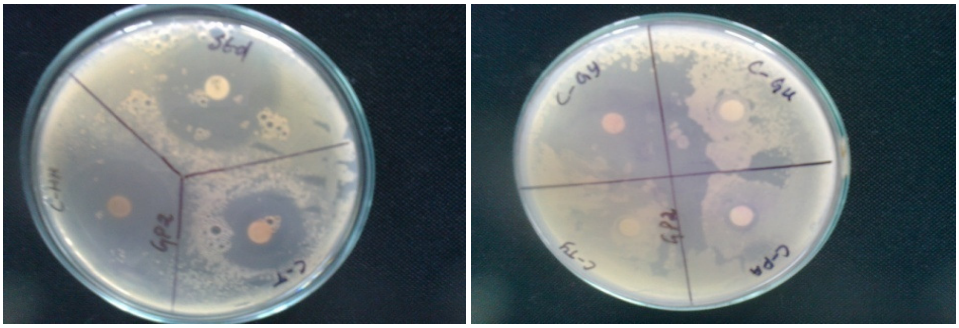
S.No	Micro organism	MIC VALUES (µg/ml)									

1.	*M.luteus*	1.25	5	5	10	10	1.25	5	5	1.25	1.25
2.	*S.aureus*	1.25	2.5	2.5	2.5	10	2.5	1.25	1.25	1.25	1.25
3.	*B.subtilis*	1.25	2.5	2.5	5	10	1.25	2.5	2.5	1.25	1.25
4.	*C.diphtheriae*	1.25	10	10	5	10	1.25	5	5	5	1.25
5.	*E.coli*	2.5	5	1.25	10	2.5	1.25	2.5	2.5	2.5	1.25
6.	*P.aureginosa*	1.25	5	10	2.5	5	10	2.5	2.5	1.25	1.2 5
7.	*R.rubrum*	5	10	5	1.25	5	1.25	10	10	2.5	1.25
8.	*V.cholerae*	2.5	5	1.25	10	10	1.25	5	5	2.5	1.25

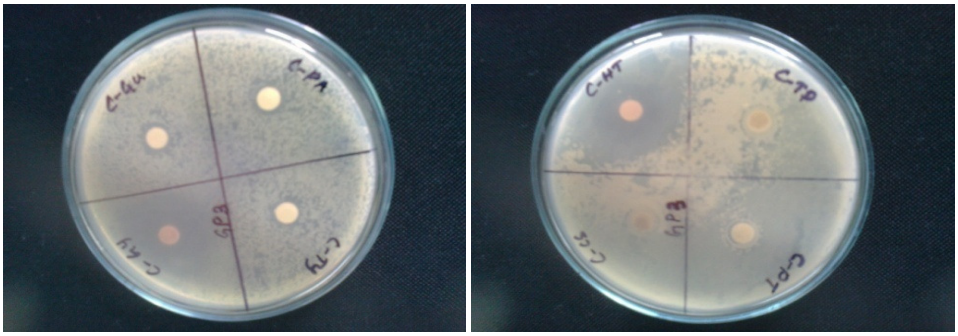
***Anti bacterial screening*:**



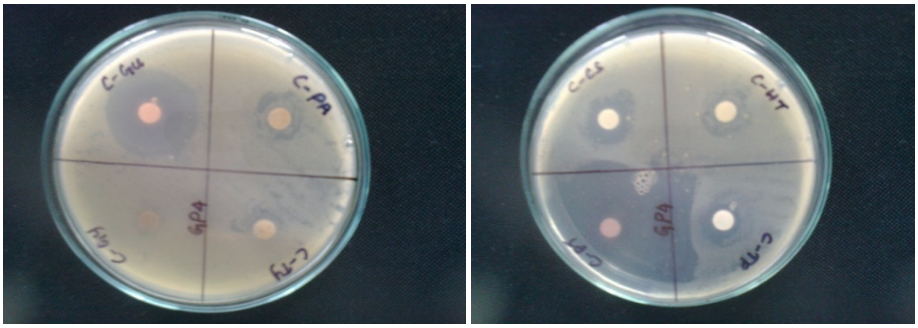
Micrococcus luteus



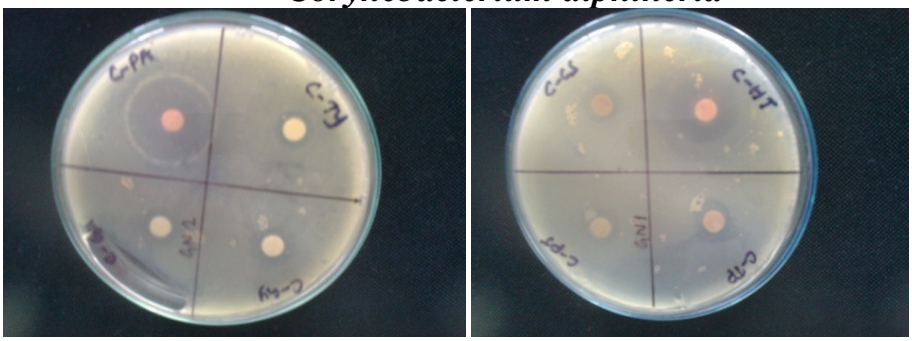
Staphylococcus aureus



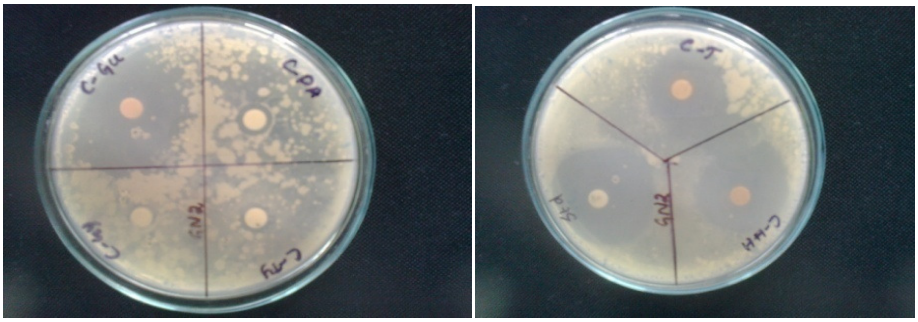
Basillus subtitiles



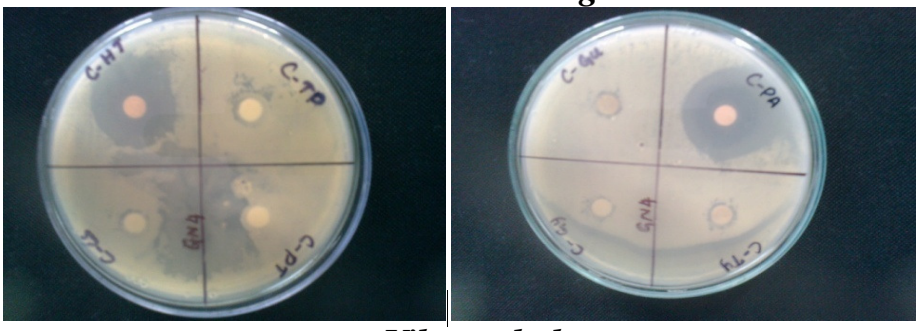
Corynebacterium diphtheria



Eschercia coli



Pseudomonas aureginosa



Vibreo cholera

Antifungal screening

Disc diffusion method

S.No	Micro organism	Zone of Inhibition										
		Compound (10µg/disc)										
		C-GY	C-GU	C-PA	C-TY	C-CY	C-HS	C-TP	C-PT	C-T	C-HH	STD Clotrimazole (10µg/disc)
1.	Candida albicans	6	5	-	3	3	-	4	3	3	-	6
2.	Streptomyces Gresus	4	5	3	4	-	5	4	-	-	-	7
3.	Aspergillus Niger	5	-	4	3	8	5	6	4	-	-	5
4.	Aspergillus Fumigalis	4	7	3	5	3	-	3	6	-	-	5

5.	Monasus ruber	-	4	-	5	3	6	4	4	-	-	6

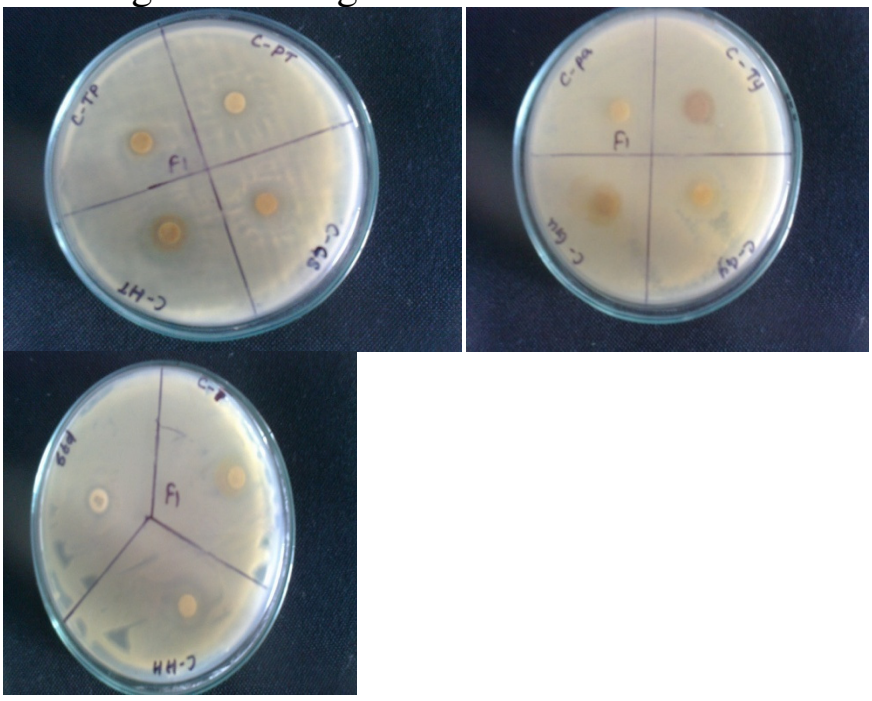
SERIAL DILUTION METHOD

MIC Values of the synthesized compounds

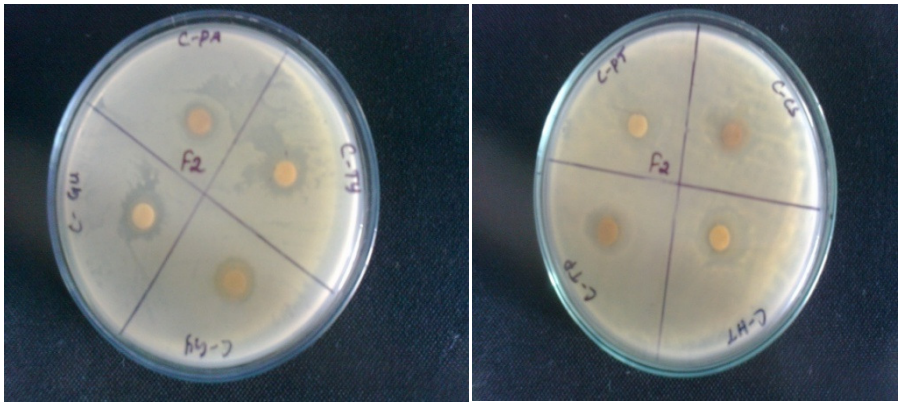
S.No	Micro organism	MIC Values (µg/ml)									
		C-GY	C-GU	C-PA	C-TY	C-CY	C-HS	C-TP	C-PT	C-T	C-HH
1.	*Candida albicans*	1.25	1.25	5	10	10	5	2.5	10	10	5
2.	*Streptomyces Gresus*	2.5	1.25	10	2.5	5	2.5	2.5	5	5	5
3.	*Aspergillus Niger*	2.5	5	2.5	10	1.25	2.5	1.25	2.5	5	5

4.	*Aspergillus Fumigalis*	2.5	1.25	10	2.5	10	5	10	1.25	5	5
5.	*Monasus ruber*	5	2.5	5	2.5	10	1.2	2.5	2.5	5	5

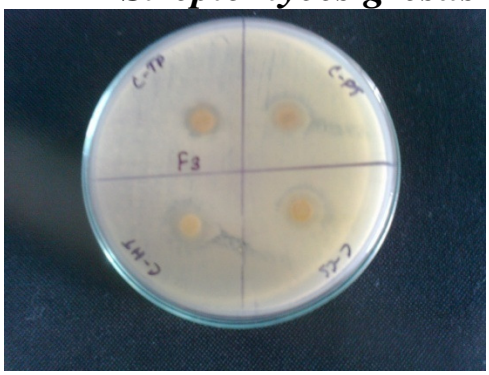
Antifungal screening:



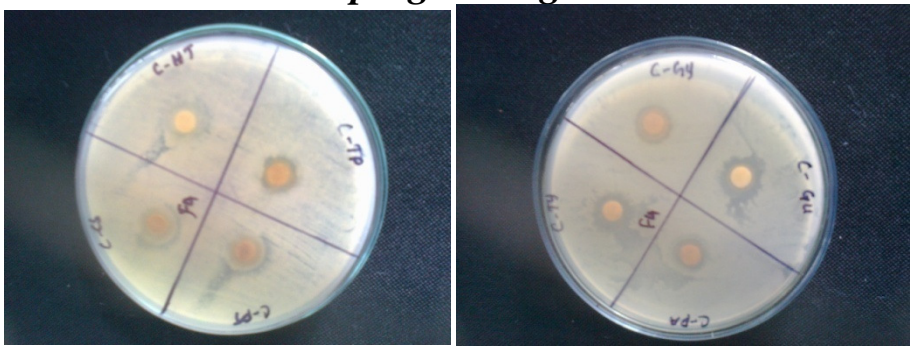
Candida albicans



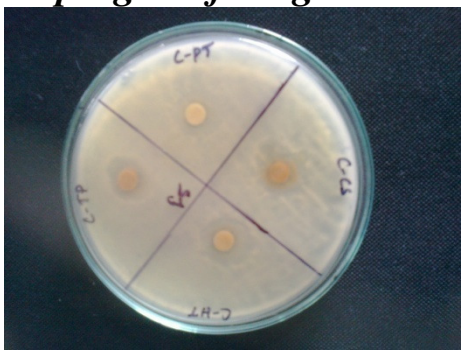
Streptomyces gresus



Aspergilus niger



Aspergilus fumigalis



Monococus ruberum

Result and Discussion

Interaction of p- chloro phenyl acetic acid with phosphorous penta chloride afforded phenyl acetyl chloride which on reaction with anthranilic acid and pyridine yields corresponding 2-chloro benzyl 1,3-benzoxazine-4-one derivative.

Reaction of compound with para amino benzoic acid in glacial acetic acid afforded 4-[2-(4-chlorobenzyl)-4-oxoquinazolinone-3(4H)-yl]benzoic acid. This was subsequently reacted with thionyl chloride, to give 4-[2-(4-chlorobenzyl)-4-oxoquinazolinone-3(4H)-yl] benzoyl derivativies. After the reaction compound added amino acids and sulfonylhydrazide compounds.

The physical parameters like melting point, molecular weight, of the synthesized compounds were determined.

The melting points of the synthesized compounds determined and were uncorrected, and melting range was found to be between, 185-298ºC

The structure of the synthesized compound was confirmed by IR, MASS and NMR spectral analysis.

ANTI – OXIDANT ACTIVITY

The synthesized compounds were tested for anti-oxidant activity by DPPH and FRAP assay method at the concentration of 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml in DMSO. Ascorbic acid was used as standard. Compounds C-TY, C-GU, C-GY and C-TP were shown good anti oxidant activity with EC- 50 value of 35, 27, 28 and 26 µg.

ANTI-CANCER ACTIVITY

The *In vitro* anticancer studies were performed on 4 selected compounds using MTT assay against HeLa cell line (NCCS). The results indicated that among the four compounds tested, the compound **C-GU and C-T** only inhibited the proliferation of cancer cells.

Antibacterial activity:

The anti-bacterial studies could be seen that all the synthesized derivatives showed moderate to good activity against the used gram(+ve) and gram(-ve) bacteria.

The MIC of the synthesized compounds against gram (+ve) and gram (-ve) bacteria determined by serial dilution method, was found to be in the range of 1.25 – 5 µg/ml.

Antifungal activity :

The anti-fungal studies showed that synthesized Quinazolinone derivatives exhibits moderate to good anti-fungal activity against organisms, with zone of inhibition in the range of 2-7mm.

In that the compound C-HH,C-T,C-GY,C-HS ,C-GU showed good activity with the zone of inhibition in the range of 8mm.

The MIC of the synthesized compounds against microorganisms against organisms determined by serial dilution method, was found to be in the range of 1.25 - 5µg/ml.

Chapter 7

Conclusion

CONCLUSION

Some novel 2,3-disubstituted-3H-Quinazolin-4-one derivatives with the aim and to get more potent drug for the treatment of Anti tumor and microbial infectious diseases.

The structure of the compounds was confirmed by spectral analysis. The synthesized 2,3-disubstituted-3H-Quinazolin-4-one derivatives exhibited moderate to good Anti oxidant, Anti tumor and Anti-microbial activity. Among those Compounds C-GY, C-GU, C-TP, and C-TY were found to be the most potent compound with Promising Anti-Oxidant activity. C-GU, C-T were found to exhibit good Anti-Cancer activity and Compound C-GY, C-HS, and C-HH were found to be a good Anti Microbial agent. Further studies on its possible mechanism and *in vivo* trials in experimental animals to broaden their Pharmacological assessment, may provide a new analogue that can overcome the side effects of existing anti tumor agents .

Chapter 8

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